[Appendix 5]

General Tests
(Related to Article 2 Subparagraph 5)

General Tests

General Tests

Acid-neutralizing Capacity
제산력시험법

The Acid-neutralizing Capacity determines the acid-neutralizing capacity of a pharmaceutical ingredient or preparation that reacts with stomach acid to neutralize its effect. When conducted according to the procedure below, the acid-neutralizing capacity of a pharmaceutical ingredient is expressed as the volume (mL) of 0.1 mol/L hydrochloric acid consumed per gram of the ingredient. For preparations, it is expressed as the volume (mL) of 0.1 mol/L hydrochloric acid consumed per daily dose. When there is a range in the daily dose, use the minimum dose for this calculation.

Preparation of sample

Solid preparations that comply with the General Requirements for Pharmaceutical ingredients and Preparations are tested as they are. For preparations in dose-unit packages, take at least 20 sachets. Weigh the contents of each sachet accurately to determine the average mass corresponding to the daily dose. Combine and uniformly mix the contents to prepare the sample. For granules and other solid preparations in unit-dose packages that do not comply with the provisions of general rules for powders, take at least 20 sachets. Weigh the contents of each sachet accurately calculate the average mass corresponding to the daily dose. Powder and combine the contents, then use the resulting powder as the sample. For granules and other solid preparations not in unit-dose packages, take at least 20 doses, powder them, and use the resultant powder as the sample. For capsules and tablets, take at least 20 doses, weigh them accurately to determine the average mass corresponding to the daily dose, powder them, and then use the resultant powder as the sample. Liquid preparations should be well-shaken before being used as the sample.

Procedure

Unless otherwise specified, a sample of 20 to 30 mL of a is taken from the following formula and tested. For pharmaceutical ingredients or solid preparations, accurately weigh the sample and place it in a 200 mL flask with a stopper. Add exactly 100 mL of 0.1 mol/L hydrochloric acid. Seal the flask with its stopper, shake for one hour at 37 ± 2 °C and then filter the solution afterward. If gas forms when adding hydrochloric acid add it carefully and, ensure that the flask is tightly sealed. Once the filtrate cools, filter again if necessary. Take exactly 50 mL of the filtrate and titrate the excess hydrochloric acid using 0.1 mol/L sodium hydroxide VS (refer to pH Measurement; endpoint: pH 3.5). Repeat this process with a blank sample.

For liquid preparations, accurately measure the sample and transfer it to a 100 mL flask. Add water to achieve a 45 mL volume. Add exactly 50 mL of 0.2 mol/L hydrochloric acid to the flask while shaking and mixing. Add more water until the total volume reaches exactly 100 mL. Transfer this solution to a 200 mL flask with a stopper, wash any remaining residue with 20 mL of water, and add it to the flask. Securely seal the flask, shake for one hour at 37±2 °C, and filter the solution. Measure exactly 60 mL of the filtrate and titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (refer to pH Measurement; endpoint: pH 3.5). This step should also be replicated with a blank sample.

Acid-neutralizing capacity (amount of 0.1 mol/L hydrochloric acid consumed per gram or daily dose)

$$(mL) = (b - a)\ f \times 2 \times t\ /s$$

a: Consumed amount of 0.1 mol/L sodium hydroxide VS (mL)

b: Consumption amount of 0.1 mol/L sodium hydroxide VS in blank test (mL)

f: Normality factor of 0.1 mol/L sodium hydroxide VS

t: 1000 mg for pharmaceutical ingredients or the daily dose for preparations (expressed in mg for solid preparations and mL for liquid preparations)

s: Sample quantity (expressed in mg for pharmaceutical ingredients and solid preparations, and mL for liquid preparations)

Alcohol Determination
알코올수측정법

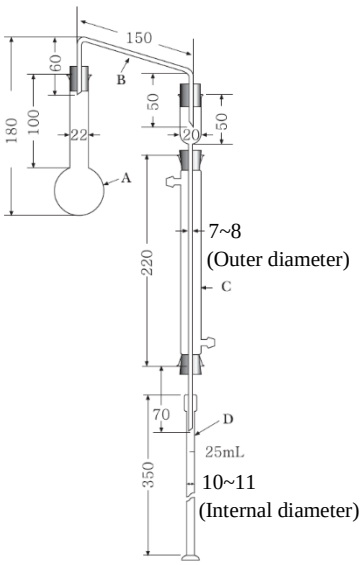
Alcohol number refers to the amount of ethanol layer (mL) obtained from 10 mL of a sample at 15 ℃ when measuring ethanol-containing preparations such as tinctures, etc. using the following method.

Method 1 Distillation

In this method, the amount of ethanol layer (mL) at 15 °C is measured after distilling 10 mL of a sample using the following method to determine the alcohol number.

1) Apparatus

Use the apparatus shown in the figure. It is entirely made of hard glass, and joints can be made of ground glass.



* The figures are in mm.

Figure

A: Distillation flask (50 mL)
B: Connector
C: Condenser
D: Stoppered measuring cylinder (25 mL, graduation: 0.1 mL)

2) Alkaline phenolphthalein

TS Dissolve 1 g of phenolphthalein in 7 mL of sodium hydroxide TS and water to make 100 mL.

3) Procedure

Take exactly 10 mL of the sample at 15 ± 2 °C, put it into a distillation flask A, add 5 mL of water and a boiling stone, carefully distill the ethanol component, and collect the distillate in a stoppered measuring cylinder D. Distill the sample, according to the ethanol content of the sample, until the approximate amount of the distillate (mL) shown in the following table is obtained. If excessive foaming occurs during distillation, add phosphoric acid or sulfuric acid to make it strongly acidic, or distill by adding a little amount of paraffin, beeswax, or silicone resin. If the sample contains glycerin, iodine, or volatile substances etc., perform the following procedures before distillation.

A) Glycerin

Add an appropriate amount of water so that the residue in the distillation flask contains at least 50% of water.

B) Iodine

Add zinc powder to bleach the sample.

C) Volatile substances

If the sample contains a significant amount of volatile oil, chloroform, ether, camphor, etc., take exactly 10 mL of the sample, put it in a separatory funnel and mix it with 10 mL of saturated sodium chloride solution. Then, add 10 mL of petroleum benzine, shake and mix, and separate the water layer. Shake and mix the petroleum benzine layer with 5 mL of saturated sodium chloride solution and repeat it twice. Add all water layers and distill it. However, at this time, depending on the ethanol content of the sample, take 2 to 3 mL more of the distillate than the amount indicated in the table.

D) Other substances

If the sample contains free ammonia, add dilute sulfuric acid to make it weakly acidic, and if it contains volatile acids, add sodium hydroxide TS to make it weakly alkaline. Also, if the sample contains both soap and volatile substances, add an excess amount of dilute sulfuric acid before adding petroleum benzine in C to decompose the soap. Add 4 - 6 g of potassium carbonate and 1 - 2 drops of alkaline phenolphthalein TS to the distillate and shake vigorously to mix it. If the water layer does not become turbid, add again an appropriate amount of potassium carbonate, mix by shaking, and leave it for 30 minutes in the water bath at 15 ± 2 °C. Read the volume of the floating red ethanol layer (mL) and set it as the alcohol number. If the surface boundary between the two liquid layers is not clear, add water dropwise, shake vigorously to mix, and observe as before.

Method 2. Gas chromatography method

A. In this method, ethanol content (vol%) is measured by taking a sample at 15 °C, performing the procedures according to the gas chromatography under the following conditions, and determining the alcohol number from the resulting value.

Table. Amount of distillate

Ethanol content in the sample (vol%)	Distillate (mL)
NLT 80	13
80 - 70	12
70 - 60	11
60 - 50	10
50 - 40	9
40 - 30	8
NMT 30	7

1) Anhydrous ethanol for alcohol number determination

It is anhydrous ethanol in which the ethanol content has been measured. However, the relationship between the specific gravity d_{15}^{15} of the anhydrous ethanol and the ethanol content is 0.797 : 99.46 vol%, 0.796 : 99.66 vol%, and 0.795 : 99.86 vol%.

2) Preparation of test and standard solutions

A) Test solution

Take exactly the amount of sample equivalent to about 5 mL of ethanol (C_2H_5OH) at 15 ± 2 °C and add water to make exactly 50 mL. Take exactly 25 mL of this solution, add exactly 10 mL of the internal standard solution and add water to make exactly 100 mL.

B) Standard solution

Take exactly 5 mL of anhydrous ethanol for alcohol number determination at a same temperature as the test solution and add water to make exactly 50 mL. Take exactly 25 mL of this solution, add exactly 10 mL of the internal standard solution and add water to make 100 mL.

3) Procedure

Take 25 mL of the test solution and the standard solution respectively, place them in a cylindrical 100 mL narrow-neck glass bottle with a rubber stopper, close it with a rubber stopper, and seal it tightly with an aluminum cap. Then, immerse the glass bottle up to the neck on a water bath, which has been left for at least 1 hour in a room with little temperature fluctuations, shake it gently to prevent the liquid from coming into contact with the stopper, and leave it to stand for 30 minutes. With 1 mL of gas in each container, perform the test according to the Gas Chromatography under the following conditions to obtain the peak height ratios of ethanol to the peak height of the internal standard, Q_T and Q_S.

$$\text{Alcohol Number} = \frac{Q_T}{Q_S} \times \frac{5\ (\text{mL})}{\text{a volume (mL) of sample}} \times \frac{V}{9.406}$$

V: Content of ethanol (C_2H_5OH) in anhydrous ethanol for alcohol number determination (vol%)

Internal standard solution—Acetonitrile solution (3 in 50).

Operating conditions

Detector: Flame ionization detector

Column: A glass column with an internal diameter of about 3 mm and a length of about 1.5 m is filled with 150 to 180 μm of porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter, 0.0075 μm; specific surface area, 500 to 600 m^2/g).

Column temperature: Constant temperature of 105 - 115 °C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: When operating under the above conditions with 1 mL of gas in the container obtained from the standard solution, use the column that elutes ethanol and the internal standard in the order, and has a resolution of 2.0 or more.

B. Measure the ethanol content (vol%) by performing procedures according to gas chromatography under the following conditions and determine the alcohol number from the resulting value.

1) Preparation of test and standard solutions

A) Test solution

Take an appropriate amount of the drug, dilute it with water to make a sample stock solution containing 2 vol% of ethanol.

Take exactly 5 mL of this stock solution and the internal standard solution respectively, mix them, and add water to make exactly 25 mL.

B) Standard solution

At the same temperature as the test solution, take exactly 5 mL of the anhydrous ethanol for alcohol number determination (2 in 100) and the internal standard solution respectively, mix them, and add water to make exactly 25 mL.

Internal standard solution—Acetonitrile solution (2 in 100).

2) Procedure

Take 0.2 - 0.5 μL of the test solution and the standard solution respectively and perform a test according to the gas chromatography under the following conditions to obtain the ratio (Q_T and Q_S) of the peak area of ethanol to that of the internal standard.

$$\text{Ethanol } (C_2H_5OH) \text{ content (vol\%)} = V \times \frac{1}{50} \times dilution\ factor \times \frac{Q_T}{Q_S}$$

V: Content of ethanol (C_2H_5OH) in anhydrous ethanol for alcohol number determination (vol%)

Dilution factor—Volume ratio of the test solution to the sample taken amount

$$\textit{Alcohol number} = \frac{Ethanol\ content\ (vol\%)}{9.460}$$

Operating conditions

Detector: Flame ionization detector

Column: A capillary column with an internal diameter of 0.53 mm and a length of about 30 m is coated with 6% cyanopropylphenyl-94% dimethylpolysiloxane gum for gas chromatography with a thickness of 3.0 μm.

Column temperature: Hold at 50 ℃ for 5 minutes, then raise the temperature by 10 ℃ per minute to 200 ℃ and hold at 200 ℃ for 4 minutes.

Sample injection port temperature: 210 ℃

Detector temperature: 280 °C

Carrier gas: Helium

Flow rate: $\frac{34cm}{seconds}$

Split ratio: About 5 : 1.

System suitability

System performance: When performing the test with the standard solution under the above conditions, the resolution between ethanol and the internal standard solution is more than 4.0 and the symmetry factor of the ethanol peak is less than 2.0.

System reproducibility: When repeating the test 6 times with the standard solution under the above conditions, the relative standard deviation of the peak area ratio of ethanol to the internal standard is less than 4.0%.

Amino Acid Analysis for Protein
단백질의 아미노산 분석법

Introduction

The Amino Acid Analysis for Protein is the method used to measure the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Proteins and peptides are macromolecules composed of amino acid residues covalently bonded together in a linear polymer. This sequence of amino acids in a protein or peptide determines the properties of the molecule. Proteins are considered large molecules that commonly exist as folded structures with a specific conformation, while peptides are smaller and may consist of only a few amino acids. Amino acid analysis can be used to quantify proteins and peptides, to determine the identity of proteins or peptides based on their amino acid composition, support protein and peptide structure analysis, evaluate fragmentation strategies for peptide mapping, and detect atypical amino acids that might be present in a protein or peptide. After hydrolysis, the amino acid analysis procedure can be similar to that employed for free amino acids in other pharmaceutical preparations. Generally, the amino acid constituents in the sample are derivatized before analysis.

Apparatus

Methods used for amino acid analysis are usually based on a chromatographic separation of the amino acids present in the sample. Modern techniques make use of specialized automated chromatographic equipment designed for analytical purposes. An amino acid analysis instrument will typically be a low-pressure or high-pressure liquid chromatograph capable of generating mobile phase gradients that separate the amino acid analytes on a chromatographic column. The instrument should have post-column derivatization capability, unless the sample is analyzed using precolumn derivatization. The detector is usually a UV-visible or fluorescence detector depending on the derivatization method used. A recording device (e.g., integrator) is used for transforming the analog signal from the detector and for quantitation. It is preferable to dedicate instrumentation specifically for amino acid analysis.

General precautions

Background contamination is always a concern for the analyst in performing amino acid analysis. High-purity reagents are necessary (e.g., low-purity hydrochloric acid can lead to glycine contamination). Analytical reagents are changed routinely every few weeks using only high-pressure liquid chromatography (HPLC) grade solvents. Potential microbial contamination and foreign materials that might be present in the solvents should be reduced by filtering solvents before use, keeping solvent reservoirs covered, and avoiding direct sunlight exposure for amino acid analysis instruments.

Laboratory practices can determine the quality of the amino acid analysis. Place the instrumentation in a low traffic area of the laboratory. Keep the laboratory clean. Clean and calibrate pipets according to a maintenance schedule. Keep pipet tips in a covered box; the analysts may not handle pipet tips with their hands. The analysts may wear powder-free latex or equivalent gloves. Limit the number of times a sample vial is opened and closed because dust can contribute to elevated levels of glycine, serine, and alanine.

A well-maintained instrument is necessary for acceptable amino acid analysis results. If the instrument is used on a routine basis, it is to be checked daily for leaks, detector and lamp stability, and the performance of column to maintain resolution of the

individual amino acids. Regularly clean or replace all instrument filters and other maintenance items according to the established schedule.

Reference material

Commercially available amino acid standards are suitable for amino acid analysis and typically consist of an aqueous mixture of amino acids. When determining amino acid composition, protein or peptide standards are alongside the test material as the blank to demonstrate the integrity of the entire procedure. Highly purified bovine serum albumin has been used as a protein standard for this purpose.

Calibration of instrumentation

Calibration of amino acid analysis instrumentation typically involves analyzing the amino acid standard, which consists of a mixture of amino acids at a number of concentrations, to determine the response factor and range of analysis for each amino acid. The concentration of each amino acid in the standard is known. In the calibration procedure, the analyst dilutes the amino acid standard to several different analyte levels within the expected linear range of the amino acid analysis technique. Then, replicates at each of the different analyte levels can be analyzed. Peak areas obtained for each amino acid are plotted versus the known concentration for each of the amino acids in the standard dilution. These results will allow the analyst to determine the range of amino acid concentrations where the peak area of a given amino acid is an approximately linear function of the amino acid concentration. It is important that the analyst prepare the samples for amino acid analysis so that they are within the analytical limits (e.g., linear working range) of the technique employed in order to obtain accurate and repeatable results.

To determine the response factor for each amino acid, four to six levels of the amino acid standard should be analyzed. The response factor is calculated as the average peak area or peak height per nmol of amino acid present in the standard. A calibration file is prepared, containing the response factor for each amino acid, which is used to calculate the concentration of each amino acid present in the sample. This calculation involves dividing the peak area corresponding to a given amino acid by the response factor for that amino acid to give the nmol of the amino acid. For routine analysis, a single-point calibration may be sufficient; however, the calibration file is updated frequently and tested by the analysis of analytical controls to ensure its integrity.

Repeatability

To obtain consistent and accurate amino acid analysis results in the laboratory, it is important to pay attention to the repeatability of the analysis. During analysis of the chromatographic separation of the amino acids or their derivatives, numerous peaks can be observed on the chromatogram that corresponds to the amino acids. The large number of peaks makes it necessary to have an amino acid analysis system that can repeatedly identify the peaks based on retention time and integrate the peak areas for quantitation. A typical repeatability evaluation involves preparing a standard amino acid solution and analyzing many replicates (i.e., six analyses or more) of the same standard solution. The relative standard deviation (RSD) is determined for the retention time and integrated peak area of each amino acid. An evaluation of the repeatability is expanded to include multiple assays conducted over several days by different analysts. These multiple assays also involve standard solution dilutions to account for variations in sample handling. Often, the amino acid composition of a standard protein (e.g., bovine serum albumin) is analyzed as part of the repeatability evaluation. By evaluating the replicate variation (i.e., RSD), the laboratory can establish analytical limits to ensure that the analyses from the laboratory are under control. It is desirable to establish the lowest practical variation limits to ensure the best results. Areas to focus on to lower the variability of the amino acid analysis include sample preparation, high background spectral interference due to the quality of reagents and/or to laboratory practices, instrument performance and maintenance, data analysis and interpretation, and analyst performance and habits. All parameters involved are fully investigated in the scope of the validation work.

Preparation of sample

Accurate results from amino acid analysis require purified protein and peptide samples. Buffer components (e.g., salts, urea, detergents) can interfere with the amino acid analysis and are removed from the sample before analysis. Methods that involve postcolumn derivatization of the amino acids are generally less affected by buffer components compared to precolumn derivatization methods. It is desirable to limit the number of sample manipulations to reduce potential background contamination, to improve analyte recovery, and to reduce labor. Common techniques used to remove buffer components from protein samples include the following methods: (1) Injecting the protein sample into a reverse-phase HPLC system, removing the protein with a volatile solvent containing a sufficient organic component, and drying the sample in a vacuum centrifuge.;(2) dialysis against a volatile buffer or water; (3) centrifugal ultrafiltration for buffer replacement with a volatile buffer or water; (4) Precipitating the protein from the buffer using an organic solvent (e.g., acetone); and (5) gel filtration.

Internal Standards

To monitor physical and chemical losses and changes during amino acid analysis, it is recommended to use internal standards. An accurately known amount of internal standard can be added to a protein solution prior to hydrolysis. The recovery of the internal standard gives the general recovery of the amino acids from the protein solution. Free amino acids, however, do not behave in the same way as protein-bound amino acids during hydrolysis because their rates of release or destruction are variable. Therefore, the use of an internal standard to correct for losses during hydrolysis may give unreliable results. It will be necessary to take this particular point into consideration when interpreting the results. Internal standards can also be added to the mixture of amino acids after hydrolysis to correct for differences in sample application and changes in reagent stability and flow rates. Ideally, an internal standard is an unnaturally occurring primary amino acid that is commercially available and inexpensive. It should also be stable during hydrolysis, its response factor should be linear with concentration, and it needs to elute with a unique retention time without overlapping other amino acids. Commonly used amino acid standards include norleucine, nitrotyrosine, and α-aminobutyric acid.

Protein Hydrolysis

For amino acid analysis of proteins and peptides, it is necessary to perform hydrolysis on the samples. To ensure accurate results, the glassware used for hydrolysis should be meticulously cleaned to avoid any potential sources of contamination. Glove powders and fingerprints on hydrolysis tubes may cause contamination. To clean glass hydrolysis tubes, they should be boiled for 1 hour in 1 N hydrochloric acid, or soaked in concentrated nitric acid, or a mixture of concentrated hydrochloric acid and nitric acid in a 1:1 ratio. Clean hydrolysis tubes are rinsed with high-purity water followed by a rinse with HPLC-grade methanol,

dried overnight in an oven, and stored covered until use. Alternatively, pyrolysis of clean glassware at 500° for 4 hours may be used to eliminate contamination from hydrolysis tubes. Adequate disposable laboratory material can also be used.

Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. The acid hydrolysis technique can contribute to the variation of the analysis due to complete or partial destruction of several amino acids. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Application of adequate vacuum (less than 200 µm of mercury or 26.7 Pa) or introduction of an inert gas (argon) in the headspace of the reaction vessel can reduce the level of oxidative destruction. In peptide bonds involving isoleucine and valine, the amido bonds of Ile-Ile, Val-Val, Ile-Val, and Val-Ile are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively. The loss of tryptophan, asparagine, and glutamine during an acid hydrolysis limits its quantitation to 17 amino acids. Some of the hydrolysis techniques described are used to address these concerns. Some of the hydrolysis techniques described (i.e., *Methods 4–11*) may cause modifications to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis.

A time-course study (i.e., amino acid analysis at acid hydrolysis times of 24, 48, and 72 hours) is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. By plotting the observed concentration of labile amino acids (i.e., serine and threonine) versus hydrolysis time, the line can be extrapolated to the origin to determine the starting concentration of these amino acids. Time-course hydrolysis studies are also used with amino acids that are slow to cleave (e.g., isoleucine and valine). During the hydrolysis time course, the analyst will observe a plateau in these residues. The level of this plateau is taken as the residue concentration. If the hydrolysis time is too long, the residue concentration of the sample will begin to decrease, indicating destruction by the hydrolysis conditions.

An acceptable alternative to the time-course study is to subject an amino acid calibration standards to the same hydrolysis conditions as the sample. The amino acid in free form may not completely represent the rate of destruction of labile amino acids within a peptide or protein during the hydrolysis. This is especially true for peptide bonds that are slow to cleave (e.g., Ile-Val bonds). However, this technique will allow the analyst to account for some residue destruction. Microwave acid hydrolysis has been used and is rapid but it requires special equipment as well as special precautions. The optimal conditions for microwave hydrolysis must be investigated for each individual protein/peptide sample. The microwave hydrolysis technique typically requires only a few minutes, but even a deviation of 1 minute may give inadequate results (e.g., incomplete hydrolysis or destruction of labile amino acids). Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins. [NOTE—During initial analyses of an unknown protein, experiments with various hydrolysis time and temperature conditions are conducted to determine the optimal conditions.]

METHOD 1

Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis Solution: 6 N hydrochloric acid containing 0.1% to 1.0% of phenol.

Procedure

Liquid Phase Hydrolysis: Place the protein or peptide sample in a hydrolysis tube, and dry. [NOTE—The sample is dried so that water in the sample will not dilute the acid used for the hydrolysis.] Add 200 µL of *Hydrolysis Solution* per 500 µg of lyophilized protein. Freeze the sample tube in a dry ice–acetone bath, and flame seal in vacuum. Samples are typically hydrolyzed at 110° for 24 hours in vacuum or inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g., 48 and 72 hours) are investigated if there is a concern that the protein is not completely hydrolyzed.

Vapor Phase Hydrolysis: This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimized by using vapor phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of *Hydrolysis Solution*. The *Hydrolysis Solution* does not come in contact with the sample. Apply an inert atmosphere or vacuum (less than 200 µm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110° for a 24-hour hydrolysis time. Acid vapor hydrolyzes the dried sample. Any condensation of the acid in the sample vials is minimized. After hydrolysis, dry the sample in vacuum to remove any residual acid.

METHOD 2

Tryptophan oxidation during hydrolysis is decreased by using mercaptoethanesulfonic acid (MESA) as the reducing acid.

Hydrolysis Solution: 2.5 M MESA solution.

Vapor Phase Hydrolysis: About 1 to 100 µg of the protein/peptide under test is dried in a hydrolysis tube. The hydrolysis tube is placed in a larger tube with about 200 µL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 µm of mercury or 6.7 Pa) to vaporize the *Hydrolysis Solution*. The hydrolysis tube is heated to between 170° to 185° for about 12.5 minutes. After hydrolysis, the hydrolysis tube is dried in vacuum for 15 minutes to remove the residual acid.

METHOD 3

Tryptophan oxidation during hydrolysis is prevented by using thioglycolic acid (TGA) as the reducing acid.

Hydrolysis Solution: a solution containing 7 M hydrochloric acid, 10% of trifluoroacetic acid, 20% of thioglycolic acid, and 1% of phenol.

Vapor Phase Hydrolysis: About 10 to 50 µg of the protein/peptide under test is dried in a sample tube. The sample tube is placed in a larger tube with about 200 µL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 µm of mercury or 6.7 Pa) to vaporize the TGA. The sample tube is heated to 166° for about 15 to 30 minutes. After hydrolysis, the sample tube is dried in vacuum for 5 minutes to remove the residual acid. Recovery of tryptophan by this method may be dependent on the amount of sample present.

METHOD 4

Cysteine-cystine and methionine oxidation is performed with performic acid before the protein hydrolysis.

Oxidation Solution: The performic acid is prepared fresh by mixing formic acid and 30 percent hydrogen peroxide (9:1),and incubating at ordinary temperature for 1 hour.

Procedure: The protein/peptide sample is dissolved in 20 µL of formic acid, and heated at 50° for 5 minutes; then 100 µL of the *Oxidation Solution* is added. In this reaction, cysteine is converted to cysteic acid and methionine is converted to methionine sulfone. The oxidation is allowed to proceed for 10 to 30 minutes. The excess reagent is removed from the sample in a vacuum centrifuge. This technique may cause modifications to tyrosine residues in the presence of halides. The oxidized protein can then be acid hydrolyzed using *Method 1* or *Method 2*.

METHOD 5

Cysteine-cystine oxidation is accomplished during the liquid phase hydrolysis with sodium azide.

Hydrolysis Solution: 6 N hydrochloric acid containing 0.2% of phenol, to which sodium azide is added to obtain a final concentration of 0.2% (w/v). The added phenol prevents halogenation of tyrosine.

Liquid Phase Hydrolysis: The protein/peptide hydrolysis is conducted at about 110° for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the sodium azide present in the *Hydrolysis Solution*. This technique allows better tyrosine recovery than *Method 4*, but it is not quantitative for methionine. Methionine is converted to a mixture of the parent methionine and its two oxidative products, methionine sulfoxide and methionine sulfone.

METHOD 6

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

Hydrolysis Solution: 6 N hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

Vapor Phase Hydrolysis: The protein/peptide hydrolysis is conducted at about 110° for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the *Hydrolysis Solution*. As an approach to limit variability and to compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine per mol protein. The response factors from protein/peptide hydrolysates are typically about 30% lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

METHOD 7

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

Reducing Solution: Transfer 83.3 µL of pyridine, 16.7 µL of 4-vinylpyridine, 16.7 µL of tributylphosphine, and 83.3 µL of water to a suitable container, and mix.

Procedure: Add the protein/peptide (between 1 and 100 µg) to a hydrolysis tube, and place in a larger tube. Transfer the *Reducing Solution* to the large tube, seal in vacuum (about 50 µm of mercury or 6.7 Pa), and incubate at about 100° for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylation reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine per mol protein to improve accuracy in the pyridylethyl-cysteine recovery. Longer incubation times for the pyridylethylation reaction can cause modifications to the α-amino terminal group and the ε-amino group of lysine in the protein.

METHOD 8

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase pyridylethylation reaction.

Stock Solutions: Prepare and filter three solutions: 1 M tris hydrochloride buffer solution (pH 8.5) containing 4 mM edetate disodium (*Stock Solution 1*), 8 M guanidine hydrochloride (*Stock Solution 2*), and 10% of 2-mercaptoethanol in water (*Stock Solution 3*).

Reducing Solution: Prepare a mixture of *Stock Solution 2* and *Stock Solution 1* (3:1) to obtain a buffered solution of 6 M guanidine hydrochloride in 0.25 M tris hydrochloride buffer solution.

Procedure: Dissolve about 10 µg of the sample in 50 µL of the *Reducing Solution*, and add about 2.5 µL of *Stock Solution 3*. Store under nitrogen or argon for 2 hours at ordinary temperature in the dark. To achieve the pyridylethylation reaction, add about 2 µL of 4-vinylpyridine to the protein solution, and incubate for an additional 2 hours at ordinary temperature in the dark. The protein/peptide is desalted by collecting the protein/peptide fraction from a reverse-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis.

METHOD 9

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase carboxymethylation reaction.

Stock Solutions: Prepare as directed for *Method 8*.

Carboxymethylation Solution: Prepare a solution containing 100 mg of iodoacetamide per mL of ethanol (95).

Buffer Solution: Use the *Reducing Solution*, prepared as directed for *Method 8*.

Procedure: Dissolve the sample in 50 µL of the *Buffer Solution*, and add about 2.5 µL of *Stock Solution 3*. Store under nitrogen or argon for 2 hours at ordinary temperature in the dark. Add the *Carboxymethylation Solution* in a 1.5 fold ratio per total theoretical content of thiols, and incubate for an additional 30 minutes at ordinary temperature in the dark. [NOTE—If the thiol content of the protein is unknown, then add 5 µL of 100 mM iodoacetamide for every 20 nmol of protein present.] The reaction is stopped by adding excess of 2-mercaptoethanol. The protein/peptide is desalted by collecting the protein/peptide fraction from a reverse-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis. The *S*-carboxyamidomethylcysteine formed will be converted to *S*-carboxymethyl-cysteine during acid hydrolysis.

METHOD 10

Cysteine-cystine is reacted with dithiodiglycolic acid or dithiodipropionic acid to produce a mixed disulfide. [NOTE—The choice of dithiodiglycolic acid or dithiodipropionic acid depends on the required resolution of the amino acid analysis method.]

Reducing Solution: a solution containing 10 mg of dithiodiglycolic acid (or dithiodipropionic acid) per mL of 0.2 M sodium hydroxide.

Procedure: Transfer about 20 µg of the sample to a hydrolysis tube, and add 5 µL of the *Reducing Solution*. Add 10 µL of isopropyl alcohol, and then remove all of the sample liquid by vacuum centrifugation. The sample is then hydrolyzed using *Method 1*. This method has the advantage that other amino acid residues are not derivatized by side reactions, and the sample does not need to be desalted prior to hydrolysis.

METHOD 11

During acid hydrolysis, asparagine and glutamine are converted into aspartic acid and glutamic acid, respectively, making their measurement challenging. Asparagine and aspartic acid residues are added and represented by Asx, while glutamine and glutamic acid residues are added and represented by Glx. When proteins/peptides react with bis(1,1-trifluoroacetoxy)iodobenzene (BTI) during acid hydrolysis, asparagine and glutamine are transformed into diaminopropionic acid and diaminobutyric acid residues, respectively, upon acid hydrolysis. These conversions allow the analyst to determine the asparagine and glutamine content of a protein/peptide in the presence of aspartic acid and glutamic acid residues.

Reducing Solutions: Prepare and filter three solutions: a solution of 10 mM trifluoroacetic acid (*Solution 1*), a solution of 5 M guanidine hydrochloride and 10 mM trifluoroacetic acid (*Solution 2*), and a freshly prepared solution of N,N-dimethylformamide containing 36 mg of BTI per mL (*Solution 3*).

Procedure: In a clean hydrolysis tube, transfer about 200 µg of the sample, and add 2 mL of *Solution 1* or *Solution 2* and 2 mL of *Solution 3*. Seal the hydrolysis tube in vacuum. Heat the sample at 60° for 4 hours in the dark. The sample is then dialyzed with water to remove the excess reagents. Extract the dialyzed sample three times with equal volumes of *n*-butyl acetate, and then lyophilize. The protein can then be acid hydrolyzed using previously described procedures. The α-, β-diaminopropionic and diaminobutyric acid residues do not typically resolve from the lysine residues upon ion-exchange chromatography based on amino acid analysis. Therefore, when using ion-exchange as the mode of amino acid separation, the asparagine and glutamine contents are the quantitative difference in the aspartic acid and glutamic acid assayed contents with underivatized and BTI-derivatized acid hydrolysis. [NOTE—The threonine, methionine, cysteine, tyrosine, and histidine assayed content can be altered by BTI derivatization; a hydrolysis without BTI will have to be performed if the analyst is interested in the composition of these other amino acid residues.]

Methodologies of amino acid analysis general principles

Many amino acid analysis techniques exist, and the choice of any one technique often depends on the sensitivity required from the assay. In general, about one-half of the amino acid analysis techniques employed rely on the separation of the free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., using ninhydrin or o-phthalaldehyde). Postcolumn detection techniques can be used with samples that contain small amounts of buffer components, such as salts and urea, and generally require between 5 and 10 µg of protein sample per analysis. On the other hand, other amino acid analysis methods involve precolumn derivatization of free amino acids (e.g., phenyl isothiocyanate; 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate or o-phthalaldehyde; (dimethylamino)azobenzenesulfonyl chloride; 9-fluorenylmethyl chloroformate; and 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole), followed by reverse-phase HPLC. Precolumn derivatization techniques are very sensitive and usually require between 0.5 and 1.0 µg of protein sample per analysis but may be influenced by buffer salts in the samples. Precolumn derivatization techniques may also result in multiple derivatives of a given amino acid, which complicates the result interpretation. Postcolumn derivatization techniques are generally influenced less by performance variation of the assay than precolumn derivatization techniques.

The following *Methods* may be used for quantitative amino acid analysis. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, and chromatographic systems. Specific parameters may vary according to the exact equipment and procedure used. Many laboratories will utilize more than one amino acid analysis technique to exploit the advantages offered by each. In each of these *Methods*, the analog signal is visualized by means of a data acquisition system, and the peak areas are integrated for quantification purposes.

METHOD 1—POSTCOLUMN NINHYDRIN DETECTION GENERAL PRINCIPLE

Ion-exchange chromatography with postcolumn ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic purple or yellow color. Amino acids, except imino acids, give a purple color, and show maximum absorption at 570 nm. The imino acids, such as proline, give a yellow color, and show maximum absorption at 440 nm. The postcolumn reaction between ninhydrin and amino acid eluted from the column is monitored at 440 nm and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition. Detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for proline. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 1 µg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

METHOD 2—POSTCOLUMN OPA FLUOROMETRIC DETECTION GENERAL PRINCIPLE

o-Phthalaldehyde (OPA) reacts with primary amines in the presence of thiol compound to form highly fluorescent isoindole products. This reaction is utilized for the postcolumn derivatization in analysis of amino acids by ion-exchange chromatography. The rule of the separation is the same as *Method I*. Instruments and reagents for this form of amino acid analysis are available commercially. Many modifications of this method exist.

Although OPA does not react with secondary amines (imino acids, such as proline) to form fluorescent substances, the oxidation with sodium hypochlorite allows secondary amines to react with OPA. The procedure employs a strongly acidic cation-exchange column for separation of free amino acids followed by postcolumn oxidation with sodium hypochlorite and postcolumn derivatization using OPA and thiol compound, such as *N*-acetyl-L-cysteine and 2-mercaptoethanol. The derivatization of primary amino acids are not noticeably affected by the continuous supply of sodium hypochlorite.

Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes of pH and cation strength. After postcolumn derivatization of eluted amino acids with OPA, the reactant passes through the fluorometric detector. Fluorescence intensity of OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limit is considered to be a few tens of pmol level

for most of the amino acid derivatives. Response linearity is obtained in the range of a few pmol level to a few tens of nmol level. To obtain good compositional data, a sample greater than 500 ng before hydrolysis is best suited for the amino acid analysis of protein/peptide.

METHOD 3—PRECOLUMN PITC DERIVATIZATION

GENERAL PRINCIPLE

Phenylisothiocyanate (PITC) reacts with amino acids to form phenylthiocarbamyl (PTC) derivatives, which can be detected with high sensitivity at 254 nm. Therefore, precolumn derivatization of amino acids with PITC followed by a reverse-phase HPLC separation with UV detection is used to analyze the amino acid composition.

After the reagent is removed under vacuum, the derivatized amino acids can be stored dry and frozen for several weeks with no significant degradation. If the solution for injection is kept cold, no noticeable loss in chromatographic response occurs after three days.

Separation of the PTC-amino acids on a reverse-phase HPLC with ODS column is accomplished through a combination of changes in concentrations of acetonitrile and buffer ionic strength. PTC-amino acids eluted from the column are monitored at 254 nm.

Detection limit is considered to be 1 pmol for most of the amino acid derivatives. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, a sample larger than 500 ng of protein/peptide before hydrolysis is best suited for this amino analysis of proteins/peptides.

METHOD 4—PRECOLUMN AQC DERIVATIZATION

GENERAL PRINCIPLE

Precolumn derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by reverse-phase HPLC separation with fluorometric detection is used.

AQC reacts with amino acids to form stable, fluorescent unsymmetric urea derivatives (AQC-amino acids) which are readily amenable to analysis by reverse-phase HPLC. Therefore, precolumn derivatization of amino acids with AQC followed by reverse-phase HPLC separation is used to analyze the amino acid composition.

Separation of the AQC-amino acids on an ODS column is accomplished through a combination of changes in the concentrations of acetonitrile and salt. Selective fluorescence detection of the derivatives with excitation at 250 nm and emission at 395 nm allows for the direct injection of the reaction mixture with minimal interference from the major fluorescent reagent byproduct, 6-aminoquinoline. Excess reagent is rapidly hydrolyzed ($t_{1/2}$< 15 seconds) to yield 6-aminoquinoline, *N*-hydroxysuccinimide and carbon dioxide, and after 1 minute no further derivatization can take place.

Peak areas for AQC-amino acids are essentially unchanged for at least 1 week at ordinary temperature, and the derivatives have more than sufficient stability to allow for overnight automated chromatographic analysis.

The detection limit is considered to be ranging from about 40 fmol to 320 fmol for each amino acid, except for Cys. The detection limit for Cys is approximately 800 fmol. Response linearity is obtained in the range of 2.5 µM to 200 µM with correlation coefficients exceeding 0.999. Accurate compositional data can be obtained with samples containing at least 30 ng of protein/peptide before hydrolysis for amino acid analysis.

METHOD 5—PRECOLUMN OPA DERIVATIZATION

GENERAL PRINCIPLE

Precolumn derivatization of amino acids with *o*-phthalaldehyde (OPA) followed by reverse-phase HPLC separation with fluorometric detection is used. This technique does not detect amino acids that exist as secondary amines (e.g., proline).

OPA in conjunction with a thiol reagent reacts with primary amine groups to form highly fluorescent isoindole products. 2-Mercaptoethanol and 3-mercaptopropionic acid can be used as thiol. OPA itself does not fluoresce and consequently produces no interfering peaks. In addition, its solubility and stability in aqueous solution, along with the rapid kinetics for the reactions, make it amenable to automated derivatization and analysis using an autosampler to mix the sample with the reagent. However, lack of reactivity with secondary amino acids has been a predominant drawback. This method does not detect amino acids that exist as secondary amines (e.g., proline). To compensate for this drawback, this technique may be combined with another technique described in *Method 7* or *Method 8*.

Precolumn derivatization of amino acids with OPA is followed by reverse-phase HPLC separation. Because of the instability of the OPA-amino acid derivative, HPLC separation and analysis are performed immediately following derivatization. The liquid chromatograph is equipped with a fluorometric detector for the detection of derivatized amino acids. Fluorescence intensity of the OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

The detection limits as low as 50 fmol via fluorescence have been reported, although the practical limit of analysis remains at 1 pmol.

METHOD 6—PRECOLUMN DABS-CI DERIVATIZATION

GENERAL PRINCIPLE

Precolumn derivatization of amino acids with (dimethylamino)azobenzenesulfonyl chloride (DABS-Cl) followed by reverse-phase HPLC separation with visible light detection is used.

DABS-Cl is a chromophoric reagent employed for the labeling of amino acids. Amino acids labeled with DABS-Cl (DABS-amino acids) are highly stable and show the maximum absorption at 436 nm.

DABS-amino acids, all 19 naturally occurring amino acid derivatives, can be separated on an ODS column of a reverse-phase HPLC by employing gradient systems consisting of acetonitrile and aqueous buffer mixture. Separated DABS-amino acids eluted from the column are detected at 436 nm in the visible region.

This method can analyze the imino acids, such as proline, together with the amino acids, at the same degree of sensitivity. DABS-Cl derivatization method permits the simultaneous quantification of tryptophan residues by previous hydrolysis of the protein/peptide with sulfonic acids, such as mercaptoethanesulfonic acid, *p*-toluenesulfonic acid, or methanesulfonic acid, described for *Method 2* in *Protein Hydrolysis*. The other acid-labile residues, asparagine and glutamine, can also be analyzed by previous conversion into diaminopropionic acid and diaminobutyric acid, respectively, by treatment of protein/peptide with BTI, described for *Method 11* in *Protein Hydrolysis*.

The nonproteinogenic amino acid, norleucine, cannot be used as an internal standard in this method as this compound is eluted in a chromatographic region crowded with peaks of primary amino acids. Nitrotyrosine can be used as an internal standard because it is eluted in a clean region.

The detection limit of DABS-amino acid is about 1 pmol. As little as 2 to 5 pmol of an individual DABS-amino acid can be

quantitatively analyzed with reliability, and only 10 ng to 30 ng of the dabsylated protein hydrolysate is required for each analysis.

METHOD 7—PRECOLUMN FMOC-CL DERIVATIZATION

GENERAL PRINCIPLE

Precolumn derivatization of amino acids with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by reverse-phase HPLC separation with fluorometric detection is used.

FMOC-Cl reacts with both primary and secondary amino acids to form highly fluorescent products. The reaction of FMOC-Cl with amino acid proceeds under mild conditions, in aqueous solution, and is completed in 30 seconds. The derivatives are stable, with only the histidine derivative showing any breakdown. Although FMOC-Cl is fluorescent itself, the reagent excess and fluorescent side-products can be eliminated without loss of FMOC-amino acids.

FMOC-amino acids are separated by reverse-phase HPLC using an ODS column. The separation is carried out by gradient elution varied linearly from a mixture of acetic acid buffer, methanol, and acetonitrile (50:40:10) to a mixture of acetonitrile and acetic acid buffer (50:50), and 20 amino acid derivatives that are separated in 20 minutes. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm.

The detection limit is in the low fmol range. A linearity range of 0.1 µM to 50 µM is obtained for most amino acids.

METHOD 8—PRECOLUMN NBD-F DERIVATIZATION

GENERAL PRINCIPLE

Precolumn derivatization of amino acids with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) followed by reverse-phase HPLC separation with fluorometric detection is used.

7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) reacts with both primary and secondary amino acids to form highly fluorescent products. Amino acids are derivatized with NBD-F by heating to 60° for 5 minutes.

NBD-amino acid derivatives are separated on an ODS column of reverse-phase HPLC by employing a concentration gradient elution system consisting of acetonitrile and aqueous buffer mixture, and 17 amino acid derivatives that are separated in 35 minutes. *E*-aminocaproic acid can be used as an internal standard because it is eluted in a clean chromatographic region. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

The sensitivity of this method is almost the same as that for the precolumn OPA derivatization method (*Method 5*), excluding proline to which OPA is not reactive and might be advantageous for NBD-F against OPA.

The detection limit for each amino acid is about 10 fmol. Profile analysis was achieved for about 1.5 mg of protein hydrolysates in the final precolumn labeling reaction mixture for HPLC.

Data calculation and analysis

When measuring the amino acid content of a protein/peptide hydrolysate, it should be noted that the acid hydrolysis step destroys tryptophan and cysteine. Serine and threonine are partially destroyed by acid hydrolysis, while isoleucine and valine residues may be only partially cleaved. Methionine can undergo oxidation during acid hydrolysis, and some amino acids (e.g., glycine and serine) are common contaminants. Application of adequate vacuum (less than 200 µm of mercury or 26.7 Pa) or introduction of inert gas (argon) in the headspace of the reaction vessel during vapor phase hydrolysis can reduce the level of oxidative destruction. Therefore, quantitative results for cysteine, tryptophan, threonine, isoleucine, valine, methionine, glycine, and serine from a protein/peptide hydrolysate may vary, necessitating further scrutiny and consideration.

Calculations

Amino Acid Mole Percent:

This represents the proportion of specific amino acid residues per 100 residues in a protein. This result may be useful for evaluating amino acid analysis data when the molecular weight of the protein/peptide under investigation is unknown. This information can be used to corroborate the identity of a protein and has other applications. Diligently identify and quantify the peaks obtained as per the respective *Procedure*. Calculate the mole percent for each amino acid present in the sample by the formula:

$$\text{Amino acid (\%mol)} = (r_U / r) \times 100$$

r_u: The peak response of the amino acid under test (nmol)

r: The sum of peak responses for all amino acids present in the sample (nmol)

Comparison of the mole percent of the amino acids under test to data from known proteins can help establish or corroborate the identity of the sample protein.

Unknown Protein Samples:

This data analysis technique can be used to estimate the protein concentration of an unknown protein sample using the amino acid analysis data. Calculate the mass, in µg, of each recovered amino acid by the formula:

$$\text{Mass of amino acid (µg)} = m \times M_W / 1000$$

m: The recovered quantity of the amino acid under test (nmol)

M_W: The molecular weight, for that amino acid, corrected for the weight of the water molecule that was eliminated during peptide bond formation.

The sum of the masses of the recovered amino acids can be used to estimate the total mass of the protein analyzed after appropriate correction for partially and completely destroyed amino acids. If the molecular weight of the unknown protein is available (e.g., through SDS-PAGE analysis or mass spectrometry), the amino acid composition of the unknown protein can be predicted. Calculate the number of residues of each amino acid by the formula:

$$\text{number of amino acid residues} = m/(1000 \times M/M_{WT})$$

m: The recovered quantity of the amino acid under test (nmol)

M: The total mass of the protein (µg)

M_{WT}: The molecular weight of the unknown protein

Known Protein Samples:

This data analysis technique can be used to investigate the amino acid composition and protein concentration of a protein sample of known molecular weight and amino acid composition using the amino acid analysis data. When the composition of the protein being analyzed is known, one can exploit the fact that some amino acids are well-recovered, while other amino acid recoveries may be compromised because of

complete or partial destruction (e.g., tryptophan, cysteine, threonine, serine, methionine), incomplete bond cleavage (i.e., for isoleucine and valine), and free amino acid contamination (i.e., by glycine and serine).

Because those amino acids that are recovered best represent the protein, these amino acids are chosen to quantify the amount of protein. Well-recovered amino acids are, typically, aspartate-asparagine, glutamate-glutamine, alanine, leucine, phenylalanine, lysine, and arginine. This list can be modified based on experience with one's own analysis system. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the calculated protein content results. The protein content determined for each of the well-recovered amino acids should be evenly distributed about the mean. Discard protein content values for those amino acids that have an unacceptable deviation from the mean. Typically, a variation greater than 5% from the mean is considered unacceptable. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample. Divide the content of each amino acid by the calculated mean protein content to determine the amino acid composition of the sample by analysis.

Calculate the relative compositional error, in percentage, by the formula:

$$\text{relative compositional error (\%)} = 100m/m_S$$

m: The experimentally determined quantity per amino acid residue of the amino acid under test (nmol)

m_S: The known residue value for that amino acid.

The average relative compositional error is the mean of the absolute values of the relative compositional errors for each individual amino acids, typically excluding tryptophan and cysteine from this calculation. The average relative compositional error provides important information regarding the integrity of the amino acid analysis process. The agreement in the amino acid composition between the protein sample and the known composition can be used to corroborate the identity and purity of the protein in the sample.

4-Aminophenol in Acetaminophen-containing Drug Products
아세트아미노펜 함유 제제 중
4-아미노페놀

This chapter provides a testing method and acceptance criteria (limits) to monitor the primary degradation product of acetaminophen, 4-aminophenol, an impurity that may arise through the hydrolysis of acetaminophen.

Procedure

Test Solution preparation Accurately measure a suitable quantity of the drug product. Form a solution with 10 mg/mL of acetaminophen using a diluent, and utilize this solution as the test stock solution. If necessary, either component of the Diluent may be introduced to the drug product first, followed by addition of the other component to maintain the proportions of acetonitrile and Buffer and to achieve the appropriate final volume defined for the Diluent. Take exactly 25 mL of the sample stock solution, add the diluent to make exactly 50 mL, then filter it using a suitable 0.45 μm filter. Discard the initial 3 mL of the filtrate, and use the next filtrate as the test solution.

Standard Solution preparation Accurately weigh about 25 mg of 4-aminophenol RS, dissolve it in the diluent to make 100 mL. Pipet 5 mL of this solution and make exactly 50 mL and use this solution as the standard stock solution. Take exactly 25 mL of the Sample stock solution, add exactly 15 mL of the Standard stock solution, and make exactly 50 mL with Diluent. Filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate and use the next filtrate as the standard solution.

Perform the test with 10 μL each of the test solution and the standard solution as directed under Liquid chromatography according to the following the specified conditions. Measure the peak areas, AT and AS, of 4-aminophenol, respectively. Calculate the percentage of 4-aminophenol (C_6H_7NO) relative to acetaminophen in the portion of drug product taken: unless otherwise specified, NMT 0.15% of 4-aminophenol relative to acetaminophen.

$$\text{percentage of 4-aminophenol} = [A_T/(A_S - A_T)] \times (W_S/W_T) \times 100$$

A_T = Peak response of 4-aminophenol from the test solution

A_S = Peak response of 4-aminophenol from the standard solution

W_S = Amount of USP 4-Aminophenol RS added to the Standard solution (mg)

W_T = Amount of acetaminophen in the test solution (mg)

Diluent—Acetonitrile and Buffer (1:9).

Buffer solution—Dissolve 4.0 g of sodium citrate dihydrate and 1.5 g of anhydrous citric acid in water to make 1000 mL.

All solution preparations that contain acetaminophen or 4-aminophe-nol should be protected from light and should be stored only for as long as can be supported by solution stability data acquired during verification under actual conditions of use.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column with an internal diameter of approximately 4.6 mm and a length of 15 cm, filled with silanized silica gel for liquid chromatography. The gel includes reversed-phase alkyl chains and weak cation-exchange carboxyl groups.

Column temperature: Maintained at a constant temperature of around 30 °C.

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 10 mM phosphate buffer prepared as follows. Add 0.60 g of monobasic potassium phosphate and 0.82 g of anhydrous sodium monohydrogen phosphate to a 1-L volumetric flask. Dissolve and dilute with water to volume to a pH of 7.0.

Mobile phase B: Water

Mobile phase C: Acetonitrile

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0.0 → 5.0	90	5	5
5.0 → 7.0	90 → 10	5 → 10	5 → 80
7.0 → 7.1	10 → 90	10 → 5	80 → 5
7.1 → 10.0	90	5	5

Flow rate: 1.0 mL/min.

System suitability

System performance: Proceed with 10 μL of the standard solution under the specified conditions. The resolution between 4-aminophenol and the nearest peak should not be less than 1.0, and the tailing factor for 4-aminophenol peak should not exceed 1.5.

System repeatability: Repeat the test 6 times, each time using 10 μL of the standard solution under the specified conditions. The relative standard deviation of the peak area of 4-aminophenol is NMT 5.0%.

S/N ratio: Pipet 5 mL of Standard stock solution, make exactly 50 mL with the diluent, and use this solution as the system suitability solution. Proceed with 10 μL of the system suitability solution according to the above conditions;S/N ratio of 4-aminophemol peak is NLT 20.

Chromatographic adjustments

The retention time of 4-aminophenol can be adjusted to enhance specificity for different product matrices. Generally, increasing the organic strength or ionic strength decreases the retention time of 4-aminophenol, and increasing the pH or column temperature increases the retention time. The use of a ternary mobile phase system affords ready changes to the ionic strength (water from Solution B) and organic strength (acetonitrile from Solution C), but this can be simplified to a binary mobile phase system.

Adjustments to the chromatographic procedure may require verification or validation. And modified conditions must meet all system suitability requirements. Solution stability must be confirmed under actual conditions of use to ensure 4-aminophenol stability in both the test solution and the standard solution, as evidenced by NMT a ±10% change in the 4-aminophenol peak areas.

Ammonium
암모늄시험법

The Ammonium is a limit test for ammonium salt contained in drugs.

In the monograph, the permissible limit for ammonium (as NH_4^+) is expressed in terms of percentage in parentheses (%).

Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Figure 1. However, when applying the reduced pressure distillation method, use the apparatus shown in Figure 2. All apparatuses are composed of hard glass, and joints can be made of ground glass. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and then boiled for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Procedure

1) Preparation of test solution and control solution

Unless otherwise specified, the test solutions and the control solution are prepared as directed in the following method.

Place an amount of the sample specified in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. Add 20 mL of boric acid solution (1 in 200) as an absorbing liquid to the collector F (measuring cylinder), and immerse the lower end of the condenser into the absorbing liquid. Adjust the heating temperature to give a rate of 5 to 7 mL of distillate per minute, and distill until the distillate reaches 60 mL.

Remove the lower end of the condenser from the solution, rinse the end part with a small amount of water, add sufficient water to make 100 mL and use it as the test solution.

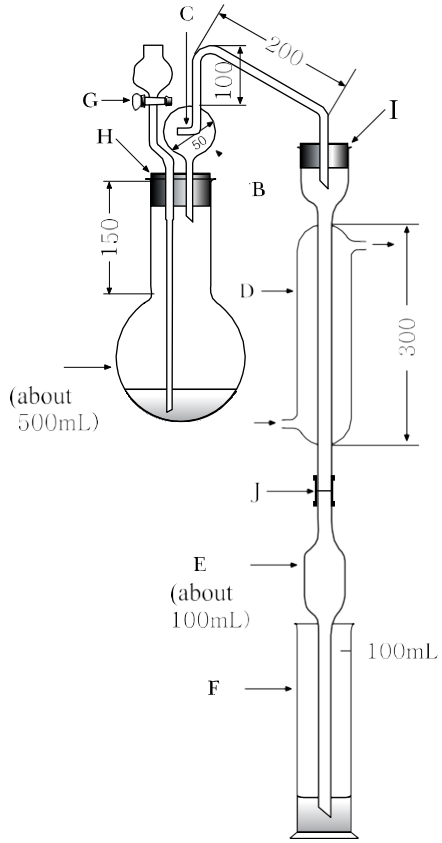
For the distillation under reduced pressure, take an amount of the sample specified in the monograph to the vacuum distillation flask L, add 70 mL of water and 1 g of magnesium oxide, and connect it to the apparatus (Figure 2). Add 20 mL of a boric acid solution (1 in 200) as an absorbing liquid to the collector M (flask), put the end of the branch tube of the distillation flask L in the absorbing liquid, and maintain the temperature at 60 °C using a water bath or an alternative equipment. Adjust the reduced pressure to get the distillate at a rate of 1 to 2 mL per minute, and distill until the distillate reaches 30 mL. Cool the collector M with running water during the distillation. Separate the absorbing liquid from the end of the branch tube, rinse in the end with a small amount of water, then add water to the liquid to make exactly 100 mL, and perform the test using this solution as the test solution.

Place an amount of the ammonium standard solution specified in the monograph in the distillation flask A or the vacuum distillation flask L, proceed as for the preparation method of the test solution, and designate it as the blank.

2) Test of the test solution and the control solution

Unless otherwise specified, proceed as directed in the following method.

Place 30 mL each of the test solution and the control solution in a Nessler tube, add 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS to each solution, and mix. Then add 4 mL of sodium hypochlorite sodium hydroxide TS and water to make 50 mL, mix , and allow to stand for 60 minutes. Compare the color of both solutions against a white background by viewing the Nessler tube downward or transversely: the color developed in the test solution is not more intense than that of the control solution.



* The figures are in mm.

Figure 1. Distilling apparatus for the Ammonium
A: Distilling flask B: Spray trap
C: Small hole D: Condenser
E: Trap F: Measuring cylinder
G: Stop cock H, I: Rubber stoppers
J: Rubber tubing

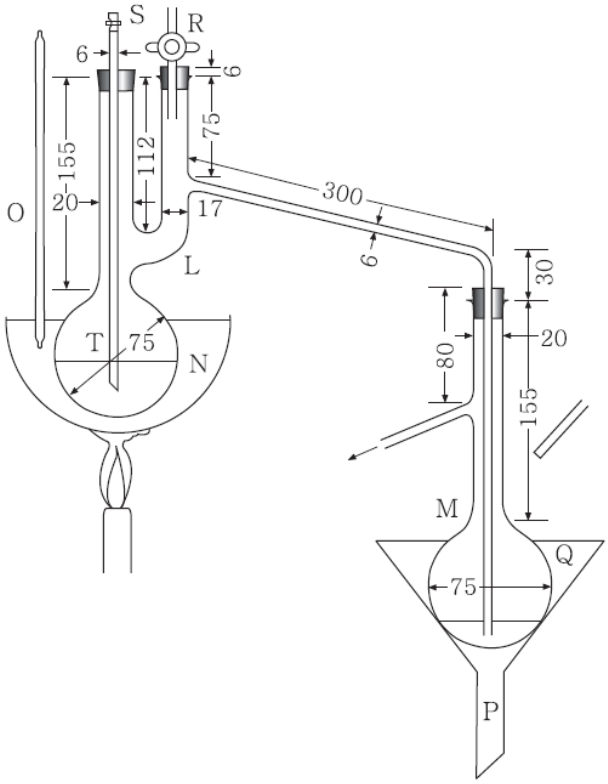


Figure 2. Vacuum distilling apparatus for the Ammonium
L: Vacuum distillation flask (200 mL)
M: Collector (a 200 mL flask) N: Water bath
O: Thermometer P: Funnel
Q: Cooling water R: Glass cock
S: Rubber tube with screw cock
T: Glass tube for anti-bumping

Analysis for Minerals
무기질시험법

1. Identification

1) Fluoride

The retention time of the major peak of the test solution corresponds to that of the standard solution, as obtained in the Assay, Method 2, for fluoride.

2) Minerals other than fluoride and iodine

The UV absorption spectra of standard solution and test solution exhibit maxima at the same wavelength, as obtained in the Assay for the corresponding mineral.

2. Assay

1) Calcium

Test solution: Prepare the test solution as follows.

Method 1: Transfer an accurately weighed portion of the sample, equivalent to about 0.3 g of calcium (Ca), into a 200-mLvolumetric flask. Add 100 mL of water, 25 mL of 6 mol/L hydrochloric acid, and 5.0 mL of polysorbate 80 TS. Heat and mix by shaking on a hot plate or on a steam bath until it is completely dissolved. After cooling, transfer the mixture to a 1-Lvolumetric flask and wash the 200-mL flask with water. Transfer the washing to the 1-L volumetric flask, dilute with water to volume,

mix by shaking and filter. Discard the first 30 mL of the filtrate, collect the next 10 mL, and dilute with 0.125 mol/L hydrochloric acid to 150 mL. Transfer 1 mL of this solution to a 100-mL volumetric flask, add 1 mL of lanthanum chloride TS and dilute with 0.125 mol/L hydrochloric acid to volume.

Method 2: Transfer an accurately weighed portion of the sample, equivalent to about 0.3 g of calcium (Ca), to a porcelain crucible. Heat the crucible in a furnace at about 550 °C for 6 to12 hours and cool. Add 15 to 20 mL of hydrochloric acid to the crucible and scrape the inner wall of the crucible with a glass rod to prevent the loss of the residue. Transfer the contents of the crucible to a 100-mL volumetric flask using the glass rod, rinse the crucible with a small amount of 6 mol/L hydrochloric acid, and add the rinsing to the flask. Dilute with 0.125 mol/L hydrochloric acid to volume, mix by shaking, and filter. Discard the first 30 mL of the filtrate, collect the next 10 mL of the filtrate, and dilute with 0.125 mol/L hydrochloric acid to 150 mL. Transfer 1 mL of this solution to a 100-mL volumetric flask, add 1 mL of lanthanum chloride TS and dilute with 0.125 mol/L hydrochloric acid to volume.

Method 3: Transfer an accurately weighed portion of the sample, equivalent to about 0.3 g of calcium (Ca), to a 100-mL volumetric flask, dilute with water to volume, mix by shaking, and filter. Discard the first 30 mL of the filtrate, collect the next 10 mL, and dilute with 0.125 mol/L hydrochloric acid to 150 mL. Transfer 1 mL of this solution to 100-mL volumetric flask, add 1 mL of lanthanum chloride TS and dilute with 0.125 mol/L hydrochloric acid to volume.

Standard solution: Accurately weigh about 1.001 g of calcium carbonate, previously dried at 300 °C for 3 hours and cooled in a desiccator for 2 hours, and dissolve in 25 mL of 1 mol/L hydrochloric acid. Dilute with water, previously boiled and cooled, to 1000 mL to obtain a standard calcium stock solution. Transfer 25 mL of the standard calcium stock solution to a 100-mL volumetric flask and dilute with 0.125 mol/L hydrochloric acid to volume to obtain an intermediate stock solution. Into separate 100-mL volumetric flasks, pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the intermediate stock solution. To each flask, add 1 mL of lanthanum chloride TS, and dilute with water to volume to obtain concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 μg/mL of calcium.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solution under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, the calcium content in the test solution can be determined.

Gas: Dissolved acetylene – nitric oxide
Lamp: Calcium hollow-cathode lamp
Wavelength: 422.7 nm
Blank: 0.125 mol/L hydrochloric acid containing 0.1% lanthanum chloride TS

2) Copper

Test solution: Accurately weigh a portion of the sample, equivalent to about 20 mg of copper (Cu), and proceed as directed in Calcium, except prepare the test solution to contain about 2 μg/mL of copper and omit the use of the lanthanum chloride TS.

Standard solution: Accurately weigh 1.0 g of copper and dissolve in a minimum volume of a 50% solution of nitric acid. Dilute with a 1% solution of nitric acid to 1 L to obtain the copper standard stock solution. Transfer 10 mL of the copper standard stock solution to a 100-mL volumetric flask and dilute with 0.125 mol/L hydrochloric acid to volume to obtain an intermediate stock solution. Into separate 200-mL volumetric flasks, pipet 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the intermediate stock solution and dilute with water to volume to obtain concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 μg/mL of copper, respectively.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the copper content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Copper hollow-cathode lamp
Wavelength: 324.7 nm
Blank: 0.125 mol/L hydrochloric acid

3) Magnesium

Test solution: Accurately weigh a portion of the sample, equivalent to about 0.2 g of magnesium (Mg), and proceed as directed for Calcium, except prepare the solution to contain about 20 μg/mL of magnesium. Transfer 2 mL of the solution and 1 mL of lanthanum chloride TS to a 100-mL volumetric flask, and dilute with 0.125 mol/L hydrochloric acid to volume.

Standard solution: Transfer 100 mg of magnesium to a 1000-mL volumetric flask, dissolve in 50 mL of 6 mol/L hydrochloric acid, and dilute with water to volume to obtain the standard magnesium stock solution. Dilute the standard magnesium stock solution with 0.125 mol/L hydrochloric acid to obtain an intermediate stock solution containing 20 μg/mL of magnesium. Into separate 100-mL volumetric flasks, pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the intermediate stock solution, add 1 mL of lanthanum chloride TS and dilute with 0.125 mol/L hydrochloric acid to volume to obtain concentrations of 0.2, 0.3, 0.4, 0.5, and 0.6 μg/mL of magnesium, respectively.

Analysis: Perform Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the magnesium content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Magnesium hollow-cathode lamp
Wavelength: 285.2 nm
Blank: 0.125 mol/L hydrochloric acid containing 0.1% lanthanum chloride TS

4) Manganese

Test solution: Accurately weigh a portion of the sample, equivalent to about 5 mg of manganese (Mn), and proceed as directed for Calcium, except prepare the test solution to contain about 1 μg/mL of manganese and omit the use of lanthanum chloride TS.

Standard solution: Accurately weigh about 1.0 g of manganese, dissolve in 20 mL of nitric acid, and dilute with 6 mol/L hydrochloric acid to 100 mL to obtain a standard magnesium stock solution. Transfer 10 mL of the standard magnesium stock solution to 100-mL and dilute with 0.125 mol/L hydrochloric acid to volume to obtain an intermediate stock solution. Dilute the intermediate stock solution with 0.125 mol/L hydrochloric acid to obtain solutions with concentrations of 0.5, 0.75, 1.0, 1.5, and 2.0 μg/mL of manganese, respectively.

Analysis: Perform Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the manganese content of the test solution.

Gas: Dissolved acetylene – air
Lamp: Manganese hollow-cathode lamp
Wavelength: 279.5 nm
Blank: 0.125 mol/L hydrochloric acid

5) Molybdenum

Method 1: Test solution: Accurately weigh a portion of the sample, equivalent to 1 mg of molybdenum (Mo), transfer to a suitable flask and dissolve in a sufficient amount of water. After adding 50 mL of 6 mol/L hydrochloric acid and 5 mL of polysorbate 80 TS, heat on a hot plate or on a steam bath until completely dissolved, with an occasional stirring. After cooling to ordinary temperature, transfer the contents to a 100-mL volumetric flask. Rinse the previously used flask with water and add the washings to the 100-mL flask. Dilute with 0.125 mol/L hydrochloric acid to volume, mix by shaking and filter. Discard the first 30 mL of the filtrate and use the rest of filtrate as the test solution.

Standard solution: Accurately weigh 1.0 g of molybdenum and dissolve in 50 mL of nitric acid, warming if necessary. After cooling, dilute with water to 1 L to obtain the standard stock solution. Transfer 10 mL of the standard stock solution to 100-mL volumetric flask, and dilute with water to volume to obtain an intermediate stock solution. Into separate 100-mL volumetric flask, pipet 2.0, 10.0, and 25.0 mL of the intermediate stock solution, and add 5 mL of perchloric acid to each flask. Gently boil the solution in each flask for 15 minutes. Cool to ordinary temperature, and dilute each with ammonium chloride solution (1 in 50) to volume to obtain concentrations of 5.0, 10.0, and 25.0 μg/mL of molybdenum.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the molybdenum content of the test solution.

Gas: Dissolved acetylene – nitric oxide

Lamp: Molybdenum hollow-cathode lamp

Wavelength: 313 nm

Blank: a mixture of ammonium chloride solution (1 in 50) and perchloric acid (20 : 1).

Method 2: Test solution: Accurately weigh a portion of the sample, equivalent to about 40 μg of molybdenum (Mo), transfer to a 200-mL beaker, and add 20 mL of nitric acid. Cover the beaker with a watch glass, and boil slowly on a hot plate. Cool to ordinary temperature. Add 60 mL of perchloric acid, cover the beaker with a watch glass, and continue the heating until digestion is complete, which is indicated when the liquid becomes colorless or pale yellow. If necessary, add more nitric acid and perchloric acid for further digestion. Evaporate the solution in the beaker to dryness. Rinse the side of the beaker and the watch glass with water, and add more water to complete 50 mL in the beaker. Gently boil the water solution for 2 - 3 minutes. Cool to ordinary temperature. Add 2 drops of methyl orange TS and neutralize with ammonium hydroxide solution (1 in 2). Add 8 mL of hydrochloric acid. Transfer the content of the beaker to a 100-mL volumetric flask, rinse the beaker with water, add the rinsing to the volumetric flask, and dilute with water to volume. After mixing by shaking, transfer 50 mL of the solution to a separatory funnel. Add 1 mL of saturated sodium fluoride solution, 0.5 mL of ferrous sulfate solution, 4 mL of potassium thiocyanate solution (1 in 5), 1.5 mL of 20% tin chloride solution, and 15 mL of isoamyl alcohol to the separatory funnel. Shake the separatory funnel for 1 minute. Allow the layers to separate, and discard the aqueous layer. Add 25 mL of diluted stannous chloride TS to the separatory funnel, and shake gently for 15 seconds. Allow the layers to separate, and discard the aqueous layer. Transfer the organic layer into a centrifuge tube, centrifuge at 2000 rpm for 10 minutes, and collect the clear supernatant to use as test solution.

Standard solution: Accurately weigh about 92 mg of ammonium molybdate tetrahydrate, dissolve in water and diluted to 500 mL to obtain a standard stock solution. Transfer 20 mL of the standard stock solution to 100-mL volumetric flask, and dilute with water to volume to obtain a solution with concentration of 20 μg/mL of molybdenum. Transfer 2 mL of this solution to a 200-mL beaker, then proceed as directed for the test solution.

Analysis: Determine the absorbances of the sample (A_T) and standard (A_S) solutions at 465 nm using isoamyl alcohol as the blank according to the Ultraviolet-visible Spectroscopy.

$$\text{Amount (mg) of molybdenum (Mo)} = \text{Amount (mg) of the reference standard (as molybdenum)} \times \frac{A_T}{A_S} \times \frac{1}{2500}$$

6) Fluoride

Note: Store all solutions in plastic containers.

Method 1: Test solution: Accurately weigh a portion of the sample equivalent to about 0.2 mg of fluoride (F), and transfer it to a 100-mL volumetric flask. Add 10 mL of 1 mol/L hydrochloric acid, 25 mL of 3 mol/L sodium acetate TS and 25 mL of sodium citrate TS, and dilute with water to volume.

Standard solution: Accurately weigh about 1.105 g of sodium fluoride, previously dried at 100 °C for 4 hours and cooled in a desiccator, and transfer to 1-L volumetric flask. Dilute with water to volume to obtain the standard stock solution with a concentration of 500 μg/mL of fluoride. Transfer 20 mL of the standard stock solution to a 100-mL volumetric flask and dilute with water to volume to obtain intermediate stock solution A (100 μg/mL of fluoride). Transfer 10 mL of the intermediate stock solution A to 100-mL volumetric flask, and dilute with water to volume to obtain intermediate stock solution B (10 μg/mL of fluoride). Into five separate 100-mL volumetric flasks, transfer 3, 5, and 10 mL of intermediate stock solution B, and 5 and 10 mL of intermediate stock solution A. To each flask, add 10 mL of 1 mol/L hydrochloric acid, 25 mL of 3 mol/L sodium acetate TS and 25 mL of sodium citrate TS. Dilute the contents of each flask with water to volume to obtain concentrations of 0.3, 0.5, 1.0, 5, and 10 μg/mL of fluoride.

Analysis: To separate plastic beakers, transfer 50 mL each of the test and standard solution. Measure the potentials (mV) of each solution under stirring with a plastic-coated stirring bar on a magnetic stirrer with a pH meter equipped with a fluoride-specific electrode and a Calomel reference electrode. Rinse the electrode between measurements, taking care to avoid damaging it.

Method 2: Test solution: Accurately weigh a portion of the sample, equivalent to about 0.1 g of fluoride (F), dissolve in water in a 100-mL volumetric flask and dilute with water to volume. Transfer 1 mL of this solution to a 200-mL volumetric flask, and dilute with water to volume.

Standard solution: Accurately weigh about 221 mg of sodium fluoride, dissolve it in water in a 100-mL volumetric flask and dilute with water to volume. Transfer 1 mL of this solution to a 200-mL volumetric flask and dilute with water to volume to obtain the standard solution with a concentration of 5 μg/mL of fluoride.

Analysis: Perform ion chromatography with 25 μL of the test and standard solutions, and measure the peak areas of fluoride from the sample (A_T) and the standard (A_S) solutions, under the following operating conditions.

$$\text{Amount (mg) of fluoride (F)} = \text{Amount (mg) of the reference standard (as fluoride)} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: Pulsed electrochemical detector

Column: Internal diameter of about 3 μm to 15 μm, surface area of 350 m^2/g or less, multi-functional resin with strong anion exchange and reverse phase maintenance functions, consisting of ethyl vinyl benzene and 55% cross-linked divinylbenzene polymer (pre-column: ATC).

Mobile phase: A mixture of 0.001 mol/L sodium hydroxide and 5% methanol (75 : 2).

7) Selenium

Note: Selenium is toxic. Handle it with care.

Method 1: Test solution: Accurately weigh a portion of the sample, equivalent to about 0.1 mg of selenium (Se), transfer it to a suitable flask, add about 12 mL of nitric acid and shake until the sample specimen is completely dissolved. Gently boil for about 15 minutes, and cool to ordinary temperature. Add 80 mL of perchloric acid to the flask, and heat the flask until the perchloric acid fumes disappear. Repeat this procedure until the fumes completely disappear. Cool to ordinary temperature. Transfer the content of the flask to a 50-mL volumetric flask. Rinse the flask with ammonium chloride solution (1 in 50), and add the rinsing to the 50-mL volumetric flask. Dilute with ammonium chloride solution (1 in 50) to volume.

Standard solution: Accurately weigh about 1.0 g of selenium, and dissolve in a minimum volume of nitric acid. Evaporate to dryness. Add 2 mL of water and evaporate to dryness. Repeat the addition of water and evaporation to dryness three times. Dissolve the residue in 3 mol/L hydrochloric acid, transfer to a 1-L volumetric flask and dilute with 3 mol/L hydrochloric acid to volume to obtain a selenium standard stock solution. Transfer 10 mL of the standard stock solution to a 100-mL volumetric flask and dilute with water to volume to obtain an intermediate stock solution. To separate 100-mL volumetric flasks, transfer 5, 10, and 25 mL of the intermediate stock solution, and add 5 mL of perchloric acid to each flask. Gently boil the solutions for 15 minutes, cool to ordinary temperature, and dilute with ammonium chloride solution (1 in 50) to volume to obtain solutions with concentrations of 5.0, 10.0, and 25.0 μg/mL of selenium, respectively.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the selenium content in the test solution.

Gas: Dissolved acetylene – air

Lamp: Selenium hollow-cathode lamp

Wavelength: 196 nm

Blank: a mixture of ammonium chloride solution (1in 50) and perchloric acid (20 : 1).

Method 2: Test solution: Accurately weigh a portion of the sample equivalent to about 0.1 mg of selenium (Se), transfer to a suitable flask, add 10 mL of nitric acid, and warm gently on a hot plate. Continue heating until the initial nitric acid reaction has subsided, then carefully add 3 mL of perchloric acid. [CAUTION-Exercise care at this stage, as the perchloric acid reaction will become vigorous.] Continue heating until white fumes of perchloric acid appear or until the digest begins to darken. Add 0.5 mL of nitric acid and resume heating, adding an additional 0.5 mL nitric acid if further darkening occurs. Digest for about 10 minutes after the first appearance of perchloric acid fumes or until the digest becomes colorless. Cool the flask, add 2.5 mL of hydrochloric acid solution, and return the flask to the hot plate to expel residual nitric acid. Heat the mixture for 3 minutes after it begins to boil. Cool the flask to ordinary temperature, and dilute with water to 20 mL.

Standard solution: Transfer a volume of the selenium standard stock solution prepared as directed in Method 1, and dilute with 0.125 mol/L hydrochloric acid to obtain a concentration of 2.0 μg/mL of selenium. Transfer 10 mL of this solution to a glass-stoppered flask. Add 1 mL of perchloric acid and 1 mL of dilute hydrochloric acid (1 in 10), and dilute with water to 20 mL

Blank test solution: Transfer 1 mL of perchloric acid and 1 mL of diluted hydrochloric acid (1 in 10) to a glass-stoppered flask, and dilute with water to 20 mL.

Analysis: Treat the test solution, standard solution, and blank test solution as follows. Add 5 mL of the reagent A to each flask, and swirl gently to mix. With ammonium hydroxide solution (1 in 2), adjust the solution in each flask to a pH of 1.1 ± 0.1. Add 5 mL of reagent B to each flask, and swirl gently to mix. Place the flasks on a water bath maintained at 50 °C, and equilibrate for 30 minutes, taking care to ensure that the flasks are covered to protect them from light. Cool to ordinary temperature, and transfer the contents of each flask to separate separatory funnels. Transfer 10 mL of cyclohexane to each separatory funnel, and extract vigorously for 1 minute. Discard the aqueous layer. Transfer the cyclohexane layer to a centrifuge tube, and centrifuge at 1000 rpm for 1 minute to remove any remaining water. Determine the absorbances of the solutions from the sample (AT) and standard (AS) solutions against the solution from the blank at 380 nm according to the Ultraviolet-visible Spectroscopy.

$$\text{Amount (mg) of selenium (Se)} = \text{Amount (mg) of the reference standard (as selenium)} \times \frac{A_T}{A_S} \times \frac{1}{50000}$$

Reagent A—Dissolve 4.5 g of sodium edetate in 400 mL of water, add 12.5 g of hydroxylamine hydrochloride, and dilute with water to 500 mL.

Reagent B—Transfer 0.2 g of 2,3-diaminonaphthalene to a 250-mL separatory funnel, and dissolve in 200 mL of 0.1 mol/L hydrochloric acid. Wash the solution with three 40-mL portions of cyclohexane, and discard the cyclohexane layer. Filter the solution into a brown bottle, and cover the solution with a 1-cm layer of cyclohexane. This solution is stable for 1 week if stored in a refrigerator.

8) Zinc

Test solution: Transfer an accurately weighed portion of the sample, equivalent to about 0.2 g of zinc (Zn), to a suitable flask, and dissolve in a sufficient amount of water. Add 25 mL of 6 mol/L hydrochloric acid and 5mL of polysorbate 80 TS. Heat and mix by shaking on a hot plate or steam bath until it is completely dissolved. After cooling, transfer the mixture to a 1-L volumetric flask, and wash the 200-mL flask with water. Add the washing to the 1-L volumetric flask, dilute with water to volume, mix by shaking, and filter. Discard the first 30 mL of the filtrate, collect a suitable volume of the next filtrate, and dilute with 0.125 mol/L hydrochloric acid to obtain the nominal concentration of 2.0 μg/mL of zinc.

Standard solution: Accurately weigh about 0.311 g of zinc oxide, dissolve in 80 mL of 5 mol/L hydrochloric acid, by heating if necessary, and allow to cool. Dilute with water to obtain a standard stock solution with a concentration of 100 μg/mL of zinc. Dilute the standard stock solution with 0.125 mol/L hydrochloric acid to obtain an intermediate stock solution with a concentration of 50 μg/mL of zinc. To separate 100-mL volumetric flasks, pipet 1, 2, 3, 4, and 5 mL of the intermediate stock solu-

tion, and dilute the contents of each flask with 0.125 mol/L hydrochloric acid to volume to obtain concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 μg/mL of zinc, respectively.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the zinc content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.8 nm
Blank: 0.125 mol/L hydrochloric acid

9) Iodine

Transfer an accurately weighed portion of the sample, equivalent to about 3 mg of iodine (I), to a nickel crucible. Add 5 g of sodium carbonate, 5 mL of sodium hydroxide solution (1 in 2), and 1 mL of ethanol, taking care to ensure that the entire specimen is moistened. Heat the crucible on a steam bath to evaporate the ethanol, then dry the crucible at 100 °C for 30 minutes to prevent spattering upon subsequent heating. Transfer the crucible with its contents to a furnace heated to 500 °C, and heat the crucible for 15 minutes (if necessary, a higher temperature may be used to ensure complete carbonization of all organic matter.) Cool the crucible, add 25 mL of water, cover the crucible with a watch glass, and boil gently for 10 minutes. Filter the solution and wash the crucible with boiling water, collecting the filtrate and washings in a beaker. Add phosphoric acid until the solution is neutral, then add another 1 mL of phosphoric acid. Add an excess amount of bromine water, boil gently until colorless, and then continue to boil for another 5 minutes. Add 2 to 3 crystals of salicylic acid, and cool the solution to 20 °C. Add 1 mL of phosphoric acid and 0.5 g of potassium iodide, and titrate the liberated iodine with 0.005 mol/L sodium thiosulfate VS, adding starch solution when the color of the liberated iodine has nearly disappeared.

Each mL of 0.005 mol/L sodium thiosulfate VS
= 105.8 μg of I

10) Phosphorus

Test solution: Transfer an accurately weighed portion of the sample, equivalent to about 10 mg of phosphorus (P), to a suitable flask. Add 25 mL of nitric acid and digest on a hot plate for 30 minutes. Add 15 mL of hydrochloric acid, and continue the digestion until the cessation of brown fumes. Cool, and transfer the contents of the flask to a 50-mL volumetric flask with the aid of small portions of water. Dilute with water to volume. Transfer 10 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Standard solution: Accurately weigh 4.395 g of potassium dihydrogen phosphate monohydrate, previously dried at 105 °C for 2 hours and stored in a desiccator, and transfer to a 1-L volumetric flask. Dissolve in water, add 6 mL of concentrated sulfuric acid, and dilute with water to volume to obtain a standard phosphorus stock solution. Dilute the standard phosphorous stock solution with water to obtain a standard solution with a concentration of 20 μg/mL of phosphorous.

Analysis: Into three separate 25-mL volumetric flasks, transfer 5 mL each of the test solution, standard solution, and water to provide the blank. To each of the three flasks, add 1 mL each of ammonium molybdate TS, hydroquinone TS, and sodium bisulfite solution (1 in 5), and swirl to mix. Dilute the contents of each flask with water to volume, and allow the flask to stand for 30 minutes. Determine the absorbances of the sample (A_T) and standard (A_S) solutions against the blank test solution at 650 nm according to the Ultraviolet-visible Spectroscopy.

Amount (mg) of phosphorus (P)
= Amount (mg) of the reference standard (as phosphorus)
$\times \frac{A_T}{A_S} \times \frac{1}{10}$

11) Iron

Test solution: Accurately weigh a portion of the sample, equivalent to about 10 mg of iron (Fe), transfer to a porcelain crucible, and proceed as directed for Calcium, except prepare the test solution to contain a nominal concentration of 5 μg/mL of iron and omit the use of lanthanum chloride TS.

Standard solution: Accurately weigh about 0.1 g of iron, dissolve it in 25 mL of 6 mol/L hydrochloric acid, and dilute with water to 100 mL to obtain a iron standard stock solution. To separate 100-mL flasks, pipet 2, 4, 5, and 8 mL of the iron standard stock solution, and dilute with water to volume to obtain concentrations of 2.0, 4.0, 5.0, 6.0, and 8.0 μg/mL of iron, respectively.

Analysis: Perform Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the iron content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Iron hollow-cathode lamp
Wavelength: 248.3 nm
Blank: 0.125 mol/L hydrochloric acid

12) Potassium

Test solution: Accurately weigh a portion of the sample, equivalent to about 0.2 g of potassium (K), transfer to a porcelain crucible, and proceed as directed for Calcium, except prepare the test solution to contain a nominal concentration of 1 μg/mL of potassium and omit the use of lanthanum chloride TS.

Standard solution: Accurately weigh about 0.1907 g of potassium chloride, previously dried at 105 °C for 2 hours and stored in a desiccator, transfer to 1-L volumetric flask, dissolve in water and dilute with water to volume to obtain a standard stock solution. Dilute the standard stock solution with 0.125 mol/L hydrochloric acid to obtain a solution with a concentration of 10 μg/mL of potassium. Into separate 100-mL flasks, pipet 5, 10, 15, 20, and 25 mL of this solution, and dilute the content of each flask with 0.125 mol/L hydrochloric acid to volume to obtain concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 μg/mL of potassium, respectively.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the potassium content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Potassium hollow cathode lamp
Wavelength: 766.5 nm
Blank: water

13) Chromium

Test solution: Accurately weigh a portion of the sample, equivalent to about 0.2 g of chromium (Cr), transfer to a porcelain crucible, and proceed as directed for Calcium, except prepare the test solution to contain 2 μg/mL of chromium and omit the use of lanthanum chloride TS.

Standard solution: Accurately weigh about 2.829 g of potassium dichromate, previously dried at 120 °C for 4 hours and cooled, and dissolve in water to obtain a standard chromium

stock solution with a concentration of 1000 μg/mL of chromium. Transfer 10 mL of the standard chromium stock solution to a 1-L volumetric flask, add 50 mL of 6 mol/L hydrochloric acid, and dilute with water to volume to obtain an intermediate stock solution. Transfer 10 mL and 20 mL of the intermediate stock solution to separate 100-mL volumetric flasks, and transfer 15 mL and 20 mL of the intermediate stock solution to separate 50-mL volumetric flasks. Dilute the content of each of the four flasks with 0.125 mol/L hydrochloric acid to volume to obtain concentrations of 1.0, 2.0, 3.0, and 4.0 μg/mL of chromium, respectively.

Analysis: Perform the Atomic Absorption Spectroscopy with the test and standard solutions under the following conditions. Based on the calibration curve obtained from the absorbance of the standard solution, determine the chromium content in the test solution.

Gas: Dissolved acetylene – air
Lamp: Chromium hollow-cathode lamp
Wavelength: 357.9 nm
Blank: 0.125 mol/L hydrochloric acid

Test Solutions

1) Sodium citrate TS: Dissolve 222 g of sodium citrate in 250 mL of water in 1-L volumetric flask. Add 28 mL of perchloric acid, and dilute with water to volume.

2) Ammonium molybdate TS: Dissolve 12.5 g of ammonium molybdate in 150 mL of water. Add 100 mL of sulfuric acid solution and mix by shaking.

3) Saturated sodium fluoride solution: Add 200 mL of water to about 10 g of sodium fluoride, shake until it becomes a saturated solution, and filter. Store the solution in a polyethylene container.

4) Bromine water: To 20 mL of bromine in a glass-stoppered bottle, add 100 mL of water. Insert the stopper into the bottle, and shake. Allow to stand for 30 minutes, and use the supernatant.

5) 20% stannous chloride TS: Transfer 40 g of stannous chloride to a beaker, add 20 mL of a mixture of hydrochloric acid and water (1 : 1.85), and heat the solution until the stannous chloride is dissolved. Cool, and dilute with water to 100 mL.

6) Dilute stannous chloride TS: Transfer 4 mL of 20% stannous solution to a 100-mL volumetric flask, and dilute with water to volume. Prepare this solution fresh at the time of use.

7) Lanthanum chloride TS: Dissolve 26.7 g of lanthanum chloride hexahydrate in 0.125 mol/L hydrochloric acid and dilute to 100 mL with the same solvent.

8) 3 mol/L Sodium acetate TS: Dissolve 408 g of sodium acetate in about 600 mL of water in a 1-L volumetric flask. Allow the solution to equilibrate to ordinary temperature, and dilute with water to volume. Adjust with 2~3 drops of acetic acid to a pH of 7.0.

9) Polysorbate 80 TS: Transfer 100 mL of polysorbate 80 to a 1-L volumetric flask and dilute with ethanol to volume.

10) Iron (II) sulfate solution: Dissolve 0.498 g of ferrous sulfate hydrate in water and dilute to 100 mL with the same solvent.

11) Sulfuric acid solution: Carefully add 37.5 mL of concentrated sulfuric acid into 100 mL of water, and mix by shaking.

12) Hydroquinone TS: Dissolve 0.5 g of hydroquinone in 100 mL of water and add 1 drop of sulfuric acid.

Analysis for Vitamins
비타민시험법

1. Identification

1) Thin layer chromatography

① Retinol acetate (Vitamin A, retinol palmitate)

Accurately weigh sample, equivalent to about 2,500 IU of retinol acetate, and transfer it to a brown flask. Add 30 mL of methanol and 3 mL of potassium hydroxide solution (1 in 2). Attach a reflux condenser and saponify the solution at 90°C for 30 minutes. After cooling, transfer the mixture to a separatory funnel, wash the flask with 20 mL of water, and add the washings to the mixture. Extract the mixture three times with 30 mL of ether, combine all the ether extracts, and add water until the washings become neutral. Dehydrate the ether extract using anhydrous sodium sulfate, evaporate the ether, dissolve the residue in 5 mL of cyclohexane, and use it as the test solution.

Separately, take an amount of sample equivalent to about 2,500 IU of retinol acetate RS. Perform the same procedures as the test solution and use it as the standard. With these solutions, perform the directed test using the Thin layer chromatography. Apply 10 μL of both the test and standard solutions to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, develop the plate with a mixture of cyclohexane, ether, and ethyl acetate (75 : 20 : 5) as the mobile phase until the solvent front has moved about 10 cm of the length of the plate, and air-dry the plate. Evenly spray antimony trichloride TS on the plate and heat it at 105 °C for 5 minutes. At this point, the R_f values and colors of the spots obtained from the test and standard solutions should be identical.

② Folic acid

Take sample equivalent to about 1 mg of folic acid, add 5 mL of hot water, dissolve it in 5 mL of dilute ammonia TS, filter, and use the filtrate as the test solution. Separately, dissolve about 10 mg of the folic acid RS in 100 mL of dilute ammonia TS and use it as the standard solution. With these solutions, perform the test directed under the Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, develop the plate with a mixture of ethyl acetate, acetic acid, and ethanol (4: 1: 1) as a mobile phase until the solvent front has moved about 10 cm of the length of the plate, and air-dry the plate. Observe the plate under ultraviolet rays (major wavelength: 365 nm) or spray potassium permanganate TS evenly on to the plate. At this time, the R_f value and colors of the spots obtained from the sample and the standard solutions should be identical.

③ Ergocalciferol (cholecalciferol)

Accurately weigh sample, equivalent to about 2,500 IU of ergocalciferol, transfer it to a brown flask, and add 30 mL of methanol and 3 mL of potassium hydroxide solution (1 in 2). Attach a reflux condenser and saponify the solution at 90°C for 30 minutes. After cooling, transfer the mixture to a separatory funnel and rinse the flask with 20 mL of water before combining. Extract three times with 30 mL of ether each time, combine all ether extracts, and wash with water until the washings reach a neutral pH. Dehydrate the ether extract with anhydrous sodium sulfate, evaporate the ether, and dissolve the residue in 5 mL of chloroform to use it as the test solution. Separately, take an amount equivalent to about 2,500 IU of ergocalciferol RS and use it as the standard solution, following the same procedure as for the test solution. With these solutions, perform the test directed under

Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography. Next, develop the plate using a mixture of cyclohexane, ether, and ethyl acetate (70: 20: 5) as a developing solvent until the solvent front has moved about 10 cm of the length of the plate, and air-dry the plate. Spray antimony pentachloride TS evenly on to the plate. At this time, the R_f value and the colors of the spots obtained from the sample and the standard solutions should be identical.

④ Thiamine nitrate (thiamine hydrochloride)

Add 20 mL of methanol to a sample equivalent to about 20 mg of thiamine nitrate, shake to mix, and filter it. Air-dry the filtrate on a water bath, dissolve the residue in 5 mL of methanol, and use it as the test solution. Separately, dissolve about 5 mg of thiamine nitrate RS in 5 mL of methanol, and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of water, pyridine, and acetic acid (100) (79: 19: 2) as a mobile phase, and air-dry the plate. Mix 15 mL of 1% aqueous potassium ferricyanide solution, 20 mL of water, and 100 mL of 15% ammonia water, spray on to the plate and heat at 110 °C for 10 minutes. Observe the plate under ultraviolet light (main wavelength: 366 nm). At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑤ Riboflavin (riboflavin sodium phosphate, riboflavin tetrabutyrate)

Dissolve sample equivalent to about 2.5 mg of riboflavin in 100 mL of acetic acid (100), filter it, and use the filtrate as the test solution. Separately, dissolve about 2.5 mg of riboflavin, riboflavin sodium phosphate and riboflavin tetrabutyrate RS in 100 mL of acetic acid (100) and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of with diluted ethyl acetate, acetic acid, and ethanol (4 : 1 : 1) as a mobile phase, and air-dry the plate. Observe the plate under ultraviolet light (main wavelength: 254 nm). At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑥ Orotic acid

Dissolve sample equivalent to about 30 mg of orotic acid in 100 mL of dimethylformamide, filter it, and use the filtrate as the test solution. Separately, dissolve about 20 mg of orotic acid RS in 20 mL of dimethylformamide and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of benzene, methanol, acetone, and acetic acid (100) (70: 20: 5: 5) as a mobile phase, and air-dry the plate. Apply cobalt nitrate solution and ammonia vapor to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑦ Pyridoxine hydrochloride

Take sample equivalent to about 10 mg of pyridoxine hydrochloride, add 5 mL of water (mL), shake to mix, filter the mixture, and use the filtrate as the test solution. Separately, dissolve 10 mg of pyridoxine hydrochloride RS in 5 mL of water and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of acetone, dichloromethane, tetrahydrofuran, and 25% ammonia water (65: 13: 13: 9) as a mobile phase, and air-dry the plate. Spray 2,6-dichloroquinone chloroimide TS on to the plate and apply ammonia vapor to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑧ Cyanocobalamin

Take sample equivalent to about 0.1 mg of cyanocobalamin, add 50 mL of acetic acid (100), shake to mix, filter the mixture, and concentrate the filtrate by evaporation to make about 1 mL. Use the resulting solution as the test solution. Separately, dissolve the cyanocobalamin RS in acetic acid (100) to obtain a concentration of 100 μg/mL and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components by a distance of about 10 cm using a mixture of methanol and water (95: 5) until the solvent front has moved about 10 cm of the length of the plate and air-dry the plate. Spray *o*-tolidine/potassium iodide TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑨ Ascorbic acid (calcium ascorbate, sodium ascorbate)

Take sample equivalent to about 15 mg of ascorbic acid, add 50 mL of ethanol (mL), shake to mix, filter the mixture, and use the filtrate as the test solution. Dissolve about 15 mg of the ascorbic acid RS in 50 mL of ethanol and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Then, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of ethanol and water (6: 1) as a mobile phase, and air-dry the plate. Spray 2,6-dichlorophenol-indophenol sodium TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑩ Nicotinic acid amide (nicotinic acid)

Dissolve sample equivalent to about 5 mg of nicotinic acid amide in 10 mL of ethanol and filter it. Use the filtrate as the test solution. Separately, dissolve 5 mg of nicotinic acid amide RS in 10 mL of ethanol and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of ethanol, water, and acetic acid (100) (60 : 10 : 1) as a mobile phase, and air-dry the plate. Observe the plate under ultraviolet rays (major wavelength: 254 nm) or spray chloroplatinic acid/potassium iodide TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑪ Tocopheryl acetate (tocopherol)

Take sample equivalent to about 5 mg of tocopheryl acetate, add 20 mL of ethanol, shake to mix, and filter the mixture. Transfer the filtrate to a saponification flask, add 5 mL of potassium hydroxide solution (1 in 2), attach a reflux condenser, and saponify the solution at 90 ° C for 1 hour. After cooling, transfer the mixture to a separatory funnel and wash the flask with 20 mL of distilled water to combine. Extract three times with 30 mL of ether each time, combine all ether extracts, and wash with until the washings reach a neutral pH. Dehydrate the ether extract with anhydrous sodium sulfate, evaporate the ether under nitrogen, and dissolve the residue in 5 mL of ethanol to use it as the test solution. Separately, weigh about 5 mg of tocopheryl acetate RS, add 20 mL of ethanol, and shake to mix. Add 5 mL of potassium hydroxide solution (1 in 2), attach a reflux condenser, and saponify the solution at 90 ° C for 1 hour. Proceed in the same manner as for the test solution and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components until the solvent front has moved about 10 cm of the length of the plate by using a mixture of cyclohexane and diethyl ether (4: 1) as a mobile phase, and air-dry the plate. Spray a mixture of 0.1% ferric chloride/ethanol solution and 0.1% α,α'-dipyridyl/ethanol solution on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑫ Calcium pantothenate (sodium pantothenate, dexapanthenol)

Accurately weigh sample equivalent to about 10 mg of calcium pantothenate and transfer it to a separatory funnel. Add 10 mL of water and dissolve by shaking. Add 10 mL of n-hexane, shake to mix, and use the aqueous layer as the test solution. Separately, weigh about 10 mg of calcium pantothenate RS, dissolve it in 10 mL of water, and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components in a migration distance of about 10 cm by using a mixture of ethanol, water, and acetic acid (100) (120: 20: 2) as a mobile phase, and air-dry the plate. Spray ninhydrin TS evenly on to the plate and heat at 105 °C for 10 minutes. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑬ Pyridoxal phosphate

Take sample equivalent to about 10 mg of pyridoxal phosphate, dissolve it in 5 mL of methanol, and filter it. Use the filtrate as the test solution. Separately, dissolve 10 mg of pyridoxal phosphate RS in 5 mL of methanol and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components in a migration distance of about 10 cm by using a mixture of acetone, methanol, water, and 28% ammonia water (40 : 40 : 20 : 3.5) as a mobile phase, and air-dry the plate. Observe the plate under ultraviolet light (main wavelength: 254 nm). At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑭ Thiamine disulfide

Take sample equivalent to about 50 mg of thiamine disulfide and extract it with 50 mL of ethyl acetate. Filter it and use the filtrate as the test solution. Separately, dissolve 10 mg of thiamine disulfide RS in 10 mL of ethyl acetate and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate made of silica gel for the Thin layer chromatography. Next, separate the components by a distance of about 10 cm by using a mixture of water, n-butanol, and acetic acid (100) (5: 4: 1) as a mobile phase, and air-dry the plate. Spray chloroplatinic acid/potassium iodide TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑮ DL-Carnitine hydrochloride

Dissolve sample equivalent to about 100 mg of DL-carnitine hydrochloride in 100 mL of water and filter it. Use the filtrate as the test solution. Separately, dissolve 10 mg of DL-carnitine hydrochloride RS in 10 mL of water and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components by a migration distance of about 10 cm by using a mixture of water, methanol, acetic acid (100), acetone, and 28% ammonia water (70 : 20 : 5 : 5 : 3.5) as a mobile phase, and air-dry the plate. Spray Dragendorff's TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑯ Hydroxocobalamin (hydroxocobalamin acetate, hydroxocobalamin hydrochloride)

Take sample equivalent to about 2.5 mg of hydroxocobalamin, dissolve it in 2 mL of water, filter it, and use the filtrate as the test solution. Separately, dissolve 2.5 mg of hydroxocobalamin RS in 2 mL of water and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components by a migration distance of about 10 cm by using a mixture of butanol, potassium phosphate monobasic, methanol, and acetic acid (36 : 36 : 10 : 8) as a mobile phase, and air-dry the plate. Spray 2% potassium hypochlorite solution on to the plate, allow to stand at ordinary temperature for about 1 hour, and prepare a saturated o-Toluidine solution with 2% acetic acid. Spray a mixture of the same volume of 0.85% potassium iodide solution before use. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑰ *β*-Carotene

Take sample equivalent to about 10 mg of β-carotene, add 30 mL of water, and extract with 50 mL of a mixture of petroleum ether and ether (1: 1). Then, evaporate it to dryness on a water bath, dissolve the residue in 10 mL of n-hexane, and use it as the test solution. Separately, dissolve 10 mg of β-carotene RS in 10 mL of n-hexane and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components by a migration distance of about 10 cm by using a mixture of cyclohexane and diethyl ether (80: 20) as a mobile phase, and air-dry the plate. Spray antimony (III) chloride TS on to the plate or observe the plate under ultraviolet light (main wavelength: 254 nm). At this time, the R_f value and the color of the spots obtained

from the sample and the standard solutions should be identical.

⑱ Inositol

Take sample equivalent to about 20 mg of inositol, add 10 mL of water, agitate it, and use the filtrate as the test solution. Separately, dissolve 10 mg of inositol RS in 100 mL of water and use this solution as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with aluminum oxide (with a fluorescent indicator) and silica gel and (50: 50) for the Thin layer chromatography. Next, separate the components by a migration distance of about 10 cm by using a mixture of n-propanol, water, ethyl acetate, and 25% ammonia water (6: 3: 1: 1) as a mobile phase, and air-dry the plate. Spray a mixture of 0.1 mol/L potassium permanganate and sodium carbonate (2 in 100) (1: 1) on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑲ Choline bitartrate

Take sample equivalent to about 50 mg of choline bitartrate and extract with 50 mL of water. Centrifuge the mixture, filter the clear supernatant, and use it as the test solution. Separately, dissolve 50 mg of choline bitartrate RS in 50 mL of water and use this solution as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL each of the test and standard solutions time on to a plate coated with silica gel for the Thin layer chromatography. Next, separate the components by a migration distance of about 10 cm by using a mixture of butanol, water, and acetic acid (100) (5: 3: 2) as a mobile phase, and air-dry the plate. Spray Dragendorff's TS evenly on to the plate. At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

⑳ Fursultiamine (fursultiamine hydrochloride)

Accurately weigh sample equivalent to about 50 mg of fursultiamine and extract with 50 mL of ethyl acetate. Filter it and use the filtrate as the test solution. Separately, dissolve 50 mg of fursultiamine RS in 50 mL of ethyl acetate and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components by a migration distance of about 10 cm by using a mixture of water, n-butanol, and acetic acid (100) (5: 4: 1) as a mobile phase, and air-dry the plate. Observe the plate under ultraviolet light (main wavelength: 254 nm). At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

㉑ Benfotiamine

Take sample equivalent to about 5 mg of benfotiamine, dissolve it in 20 mL of methanol, filter it and use the filtrate as the test solution. Separately, dissolve about 5 mg of benfotiamine RS in 20 mL of methanol, and use it as the standard solution. With these solutions, perform the test directed under Thin layer chromatography. Apply 10 μL of the test and standard solutions each time on to a plate coated with silica gel for the Thin layer chromatography (with a fluorescent indicator). Next, separate the components by a migration distance of about 10 cm by using a mixture of methanol and water (7: 3) as a mobile phase, and air-dry the plate. Observe the plate under ultraviolet light (main wavelength: 254 nm). At this time, the R_f value and the color of the spots obtained from the sample and the standard solutions should be identical.

2) Liquid chromatography

When tested according to the assay described below, the test solution shows the major peak at the same retention time as the standard solution.

2. Assay

1) Nicotinic acid, nicotinic acid amide, pyridoxine hydrochloride, riboflavin, riboflavin sodium phosphate, thiamine hydrochloride, and thiamine nitrate

Method 1: Accurately weigh sample equivalent to about 10 mg of nicotinic acid ($C_6H_5NO_2$) or nicotinic acid amide ($C_6H_5N_2O$), or about 2.5 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3.HCl$), or about 2.5 mg of riboflavin ($C_6H_6N_2O$) or riboflavin sodium phosphate ($C_{17}H_{20}N_4Na_5O_9P$), or about 2.5 mg of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$) or thiamine nitrate ($C_{12}H_{17}N_5O_4S$). Transfer it to a 50 mL centrifuge tube, add 25 mL of a mixture of water, acetonitrile, and acetic acid (100) (94: 5: 1), shake to mix, and suspend it completely. Leave the mixture for 5 minutes on a water bath at 65 to 70 ℃, shake to mix, filter the mixture, and use the filtrate as the test solution. Separately, accurately weigh sample equivalent to about 0.1 g of nicotinic acid or nicotinic acid amide RS or about 25 mg of pyridoxine hydrochloride RS, or about 25 mg of riboflavin or sodium riboflavin phosphate RS, or about 25 mg of thiamine hydrochloride or thiamin nitrate RS. Then, add 180 mL of a mixture of water, acetonitrile, and acetic acid (100) (94: 5: 1) and leave it for 10 minutes on a water bath at 65 to 70 ℃ while shaking occasionally. Quickly cool the solution with ice water and leave it at ordinary temperature for 10 minutes, then add a mixture of water, acetonitrile, and acetic acid (100) (94: 5: 1) to make it up to 250.0 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 10 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of nicotinic acid } (C_6H_5NO_2) = \text{Amount (mg) of nicotinic acid RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of nicotinic acid amide } (C_6H_5N_2O) = \text{Amount (mg) of nicotinic acid amide RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of pyridoxine hydrochloride } (C_8H_{11}NO_3.HCl) = \text{Amount (mg) of pyridoxine hydrochloride RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of riboflavin } (C_6H_6N_2O) = \text{Amount (mg) of riboflavin RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of riboflavin sodium phosphate } (C_{17}H_{20}N_4Na_5O_9P) = \text{Amount (mg) of riboflavin sodium phosphate RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of thiamine hydrochloride } (C_{12}H_{17}ClN_4OS.HCl) = \text{Amount (mg) of thiamine hydrochloride RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of thiamine nitrate } (C_{12}H_{17}N_5O_4S) = \text{Amount (mg) of thiamine nitrate RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 280 nm)

Column: A stainless steel column with an internal diameter of about 3.9 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Mixture of water, ethanol, and acetic acid (100) (73: 27: 1)

Method 2: Accurately weigh sample equivalent to about 10 mg of nicotinic acid ($C_6H_5NO_2$) or nicotinic acid amide ($C_6H_5N_2O$), or about 2 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl), or about 2 mg of riboflavin ($C_6H_6N_2O$) or riboflavin sodium phosphate ($C_{17}H_{20}N_4Na_5O_9P$), or about 2 mg of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS$.HCl) or thiamine nitrate ($C_{12}H_{17}N_5O_4S$). Transfer it to a 50 mL centrifuge tube, add 25 mL of a mixture of water, acetonitrile, and acetic acid (100) (94: 5: 1), mix by shaking, and suspend it completely. Add 80 mL of diluted acetic acid (100) (1 in 400), heat it for 30 minutes while shaking occasionally, and extract. After cooling it down, add diluted acetic acid (100) (1 in 400) to make it up to 100.0 mL and filter to use it as the test solution. Separately, accurately weigh about 0.1 g of nicotinic acid or nicotinic acid amide RS, or about 20 mg of pyridoxine hydrochloride RS, or about 20 mg of riboflavin or sodium riboflavin phosphate RS, or about 20 mg of thiamine hydrochloride or thiamin nitrate RS. Then, add 80 mL of diluted acetic acid (100) (1 in 400) and dissolve it by heating. After cooling it down, add diluted acetic acid (100) (1 in 400) to make it up to 100.0 mL. Take 10.0 mL of this solution, add dilute acetic acid (100) (1 in 400) to make it up to 100.0 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 10 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of nicotinic acid } (C_6H_5NO_2) = \text{Amount (mg) of nicotinic acid RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of nicotinic acid amide } (C_6H_5N_2O) = \text{Amount (mg) of nicotinic acid amide RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of pyridoxine hydrochloride } (C_8H_{11}NO_3.HCl) = \text{Amount (mg) of pyridoxine hydrochloride RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of riboflavin } (C_6H_{11}N_2O) = \text{Amount (mg) of riboflavin RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of riboflavin sodium phosphate } (C_{17}H_{20}N_4NaO_9P) = \text{Amount (mg) of riboflavin sodium phosphate RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of thiamine hydrochloride } (C_{12}H_{17}ClN_4OS.HCl) = \text{Amount (mg) of thiamine hydrochloride RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

$$\text{Amount (mg) of thiamine nitrate } (C_{12}H_{17}N_5O_4S) = \text{Amount (mg) of thiamine nitrate RS} \times \frac{A_T}{A_S} \times \frac{1}{10}$$

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 270 nm)

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 25 cm, filled with octadecylsilyl silica with particle size ranging from 5 to 10 μm.

Mobile phase: Solution prepared by putting 0.4 mL of triethylamine, 15.0 mL of acetic acid (100), and 350 mL of methanol into a 2000 mL volumetric flask and adding 0.008 mol/L sodium hexanesulfonate to make 2000.0 mL (preparation before use).

2) Vitamin A (retinol), retinol acetate, and retinol palmitate

Accurately weigh sample equivalent to about 25,000 IU of vitamin A ($C_{20}H_{30}O$), retinol acetate ($C_{22}H_{32}O_2$), or retinol palmitate ($C_{36}H_{60}O_2$), and add 40 mL of dimethyl sulfoxide and 60 mL of n-hexane. Shake to mix for 4 minutes and shake again on a water bath at 60 °C. Centrifuge this solution at 3000 rpm for 10 minutes and collect the n-hexane layer with a pipette. Repeat this procedure NLT three times to collect all the n-hexane layers, add n-hexane to make it up to exactly 500 mL, and use this as the test solution. Separately, weigh about 1,000 IU of vitamin A (retinol), retinol acetate, or retinol palmitate RS and dissolve by adding n-hexane to make exactly 20 mL. Use the resulting solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 40 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (IU) of vitamin A (retinol) } (C_{20}H_{30}O) = \text{Amount (IU) of vitamin A (retinol) } (C_{20}H_{30}O) \text{ RS} \times \frac{A_T}{A_S} \times 25$$

$$\text{Amount (IU) of retinol acetate } (C_{22}H_{32}O_2) = \text{Amount (IU) of retinol acetate } (C_{22}H_{32}O_2) \text{ RS} \times \frac{A_T}{A_S} \times 25$$

$$\text{Amount (IU) of retinol palmitate } (C_{36}H_{60}O_2) \quad \text{Amount (IU) of retinol palmitate } (C_{36}H_{60}O_2) \text{ RS} \times \frac{A_T}{A_S} \times 25$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 325 nm)

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 15 cm is filled with porous silica gel for liquid chromatography of 10 μm.

Mobile phase: n-Hexane

3) *β*-Carotene

Accurately weigh sample equivalent to about 1 mg of β-carotene ($C_{40}H_{56}$) and dissolve it in 10 mL of tetrahydrofuran. Then, add 60 mL of ethanol, perform ultrasonic extraction at 60 °C for 30 minutes, add ethanol to make it up to exactly 100 mL, and use the filtrate as the test solution. Separately, accurately weigh 5 mg of β-carotene RS and dissolve it in tetrahydrofuran to make exactly 50 mL. Take exactly 10 mL of this solution, add ethanol to make it up to exactly 100 mL, and use it as the standard solution. Prepare the test and standard solutions immediately before use. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of β-carotene } (C_{40}H_{56}) = \text{Amount (mg) of β-carotene } (C_{40}H_{56}) \text{ RS} \times \frac{A_T}{A_S} \times \frac{1}{5}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 450 nm)

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 15 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Mixture of tetrahydrofuran and ethanol (1: 99)

Flow rate: 0.8 mL/min

4) Benfotiamine

Accurately weigh sample equivalent to about 20 mg of benfotiamine ($C_{19}H_{23}N_4O_6PS$), add 80 mL of 0.1 mol/L sodium dihydrogen phosphate TS (pH 3.0), and perform ultrasonic extraction at 60 °C for 30 minutes. Then, add 0.1 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make it up to exactly 100 mL and use it as the test solution. Separately, accurately weigh about 20 mg of benfotiamine RS and dissolve it in 0.1 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make exactly 100 mL. Use this solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

Amount (mg) of benfotiamine ($C_{19}H_{23}N_4O_6PS$)
= Amount (mg) of benfotiamine RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 15 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Mixture of 0.5% ammonium carbonate and methanol (8: 2).

Flow rate: 1.0 mL/min

5) Biotin

Method 1: Accurately weigh sample equivalent to about 0.2 mg of biotin ($C_{10}H_{16}N_2O_3PS$) and add 3 mL of dimethyl sulfoxide to wet it. After heating at 70 ° C for 5 minutes, add 15 mL of water and perform ultrasonic extraction for 10 minutes to dissolve. Add water to the resulting solution to make it up to exactly 100 mL, filter it, and use it as the test solution. Separately, accurately weigh about 15 mg of biotin RS, add dimethylformamide to make it exactly 200 mL. Pipette 2 mL of the resulting solution, add water to make it exactly 75 mL, and use this solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 50 μL each of the test and standard solutions under the following conditions.

Amount (mg) of biotin ($C_{10}H_{16}N_2O_3PS$)
= Amount (mg) of biotin RS × $\frac{A_T}{A_S} \times \frac{1}{75}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 200 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 25 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Dissolve 85 mL of acetonitrile, 1 g of sodium perchlorate, and 1 mL of phosphoric acid in water to make 1000 mL.

Flow rate: 1.0 mL/min

When it is difficult to separate biotin when tested using Method 1, perform the test according to Method 2.

Method 2. Accurately weigh sample equivalent to about 0.1 mg of biotin, add 2 mL of 0.1 mol/L sodium hydroxide TS and 10 mL of water, and dissolve it by ultrasonic extraction at 50 °C for 15 minutes. After neutralizing the resulting solution with 0.1 mol/L nitric acid TS, add water to make it up to exactly 50 mL, centrifuge, take the clear supernatant, filter it, and use the filtrate as the test solution. Separately, accurately weigh about 10 mg of biotin RS, add 20 mL of 0.1 mol/L sodium hydroxide TS to dissolve. Neutralize the resulting solution with 0.1 mol/L nitric acid TS and add water to make it up to exactly 500 mL. Pipette 5 mL of this solution, add water to make it up to 50 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography (6-way switching valve system) with 100 μL each of the test and standard solutions under the following conditions.

Amount (mg) of biotin ($C_{10}H_{16}N_2O_3PS$)
= Amount (mg) of biotin RS × $\frac{A_T}{A_S} \times \frac{1}{100}$

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 210 nm).

Column:

Pretreatment column: A stainless steel column with an internal diameter of 4.6 mm and a length of 15 cm, filled with octadecylsilyl silica gel with a particle size of 5 μm.

Concentration column: A stainless steel column with an internal diameter of 2.0 mm and a length of 3.5 cm, filled with octadecylsilyl silica gel with a particle size of 5 μm.

Analytical column: A stainless steel column with an internal diameter of 1.5 mm and a length of 25 cm, filled with octadecylsilyl silica gel with a particle size of 5 μm.

Mobile phase:

Pretreatment column: 10 mmol/L potassium dihydrogen phosphate solution containing 0.1% phosphoric acid.

Analytical column: Mixture of 10 mmol/L Potassium dihydrogen phosphate solution containing 0.1% phosphoric acid and methanol (82: 18).

Flow rate:

Pretreatment column: 0.5 mL/min

Analytical column: 0.1 mL/min

6) Cyanocobalamin (vitamin B_{12})

Accurately weigh sample equivalent to about 0.1 mg of cyanocobalamin ($C_{63}N_{88}C_{14}O_{14}P$), add 100 mL of water, and shake for 2 minutes. Filter the resulting solution, and use the filtrate as the test solution. Separately, accurately weigh about 10 mg of cyanocobalamin RS and dissolve it in water to make it up to exactly 100 mL. Pipette 1 mL of the resulting solution, add water to make it up to exactly 100 mL, and use this solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 200 μL each of the test and standard solutions under the following conditions.

Amount (mg) of cyanocobalamin ($C_{63}N_{88}C_{O14}O_{14}P$)
= Amount (mg) of cyanocobalamin RS × $\frac{A_T}{A_S} \times \frac{1}{100}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 550 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 15 cm, filled with octadecylsilyl silica gel of 5 μm.

Mobile phase: Mixture of water and methanol (65: 35).

7) Ascorbic acid (vitamin C), calcium ascorbate, and sodium ascorbate

Accurately weigh sample equivalent to about 0.1 g of ascorbic acid ($C_6H_8O_6$), calcium ascorbate ($C_{12}H_{14}CaO_2$), or sodium ascorbate ($C_6H_7NaO_6$) and dissolve it with metaphosphoric

acid/acetic acid TS to make exactly 100 mL. Pipette 1 mL of this solution, add metaphosphoric acid/acetic acid TS to make it up to exactly 100 mL, and use this solution as the test solution. Separately, accurately weigh about 0.1 g of ascorbic acid RS, calcium ascorbate RS, or sodium ascorbate RS, operate it in the same way as the test solution, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of ascorbic acid } (C_6H_8O_6) = \text{Amount (mg) of ascorbic acid RS} \times \frac{A_T}{A_S}$$

$$\text{Amount (mg) of calcium ascorbate } (C_{12}H_{14}CaO_2) = \text{Amount (mg) of calcium ascorbate RS} \times \frac{A_T}{A_S}$$

$$\text{Amount (mg) of sodium ascorbate } (C_6H_7NaO_6) = \text{Amount (mg) of sodium ascorbate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Add water to 25 mL of methanol and 1 g of sodium 1-hexanesulfonate to make it up to 1000 mL, add 10 mL of acetic acid (100), shake to mix, and filter.

8) Ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3)

Accurately weigh an amount of sample equivalent to 4,000 IU as ergocalciferol ($C_{28}H_{44}O$) or cholecalciferol ($C_{27}H_{44}O$), add 20 mL of n-hexane, shake to mix, and filter. Repeat this procedure twice with 15 mL of n-hexane, combine all the filtrate and dissolve it in n-hexane to make the final concentration of ergocalciferol or cholecalciferol 80 IU/mL. Use the resulting solution as the test solution. Separately, accurately weigh about 400,000 IU of ergocalciferol or cholecalciferol RS and dissolve in n-hexane to make exactly 100 mL. Pipette 2.0 mL of the resulting solution, add n-hexane to make it up to exactly 100 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 10 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (IU) of ergocalciferol } (C_{28}H_{44}O) = \text{Amount (IU) of ergocalciferol RS} \times \frac{A_T}{A_S} \times \frac{1}{100}$$

$$\text{Amount (IU) of cholecalciferol } (C_{27}H_{44}O) = \text{Amount (IU) of cholecalciferol RS} \times \frac{A_T}{A_S} \times \frac{1}{100}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 265 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 15 cm, filled with porous aminopropylsilyl silica gel for liquid chromatography with a particle size of 10 μm.

Mobile phase: Mixture of n-hexane and isopropanol (99: 1).

9) Folic acid

Accurately weigh 20 mg of sample equivalent to folic acid ($C_{19}H_{19}N_7O_6$) and add the mobile phase to make it up to exactly 100 mL. To 2 mL of the resulting solution, add the internal standard solution to make it up to exactly 25 mL, shake for 10 minutes, centrifuge, and use the clear supernatant as the test solution. Separately, accurately weigh about 20 mg of folic acid RS and dissolve it with the mobile phase to make exactly 100 mL. Pipette 2 mL of this solution, add the internal standard solution to make it up to exactly 25 mL, and use this solution as the standard solution. Take 15 μL each of the test and standard solutions and perform the test according to liquid chromatography under the following conditions. Calculate the peak area ratio Q_T and Q_S of each solution to the peak area of the internal standard solution.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = \text{Amount (mg) of folic acid RS} \times \frac{Q_S}{Q_T}$$

Internal standard solution—Weigh about 40 mg of methyl parahydroxybenzoate and dissolve it in 220 mL of methanol. Add 300 mL of potassium dihydrogen phosphate solution (2 in 30), 19 mL of 25% tetrabutylammonium hydroxide/methanol solution, and 30 mg of a solution in which 5 g of pentetic acid is dissolved in 1 mol/L sodium hydroxide to make 50 mL. Adjust the pH to 9.8 and add water to make it up to 1 L.

Operating conditions

Detector: UV spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column with an internal diameter of about 3.0 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Dissolve 2 g of potassium dihydrogen phosphate in 650 mL of water, add 12 mL of 25% tetrabutylammonium hydroxide/methanol solution, 7 mL of 3 mol/L phosphoric acid, and 240 mL of methanol, and cool to ordinary temperature. Then, adjust the pH to 7.0 with phosphoric acid and ammonia TS and add water to make it up to 1 L.

10) Inositol

Accurately weigh sample equivalent to about 1 mg of inositol ($C_6H_{12}O_6$) and put it in a stoppered test tube. After completely drying in a freeze-dryer or vacuum-dryer (60 ℃, phosphorus pentoxide), add 3 mL of a mixture of trimethylchlorosilane, hexamethyldisilazane, and pyridine (1: 1: 1), cover with Teflon tape, and seal. Leave this solution in an oven at 80 to 90 ℃ for 2 hours while shaking occasionally. Make sure that this solution does not absorb moisture and use it as the test solution. Separately, accurately weigh about 0.1 g of inositol RS and dissolve it with water to make exactly 100 mL. Take exactly 1 mL of this solution, transfer it to a stoppered test tube, and use it as the standard solution by following the same procedure as the test solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under gas chromatography with 2 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of inositol } (C_6H_{12}O_6) = \text{Amount (mg) of inositol RS} \times \frac{A_T}{A_S} \times \frac{1}{100}$$

Operating conditions

Detector: Hydrogen flame ionization detector

Column: White clay of 80 to 100 mesh for gas chromatography made by sintering sodium carbonate on diatomaceous earth as a flux at 900 ℃ or higher is coated with 3.0% silicone SE-30 (1/8×2 m, glass).

Column temperature: 220 °C for the first 2 minutes, then

increased by 2 °C per minute.

Inlet temperature: 270 °C
Detector temperature: 260 °C
Carrier gas: Nitrogen
Flow rate: 40 mL/min

11) Pyridoxal phosphate

Accurately weigh sample equivalent to about 30 mg of pyridoxal phosphate ($C_8H_{10}NO_6P.H_2O$), dissolve it in 0.01 mol/L hydrochloric acid by shaking vigorously, and make exactly 100 mL. Use the resulting solution as the test solution. Separately, accurately weigh about 30 mg of pyridoxal phosphate RS and dissolve it in 0.01 mol/L hydrochloric acid to make exactly 100 mL. Use the resulting solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

Amount (mg) of pyridoxal phosphate ($C_8H_{10}NO_6P.H_2O$)
= Amount (mg) of pyridoxal phosphate RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Dissolve 590 mL of methanol and 1.0 g of sodium 1-octanesulfonate in water to make 1 L, add 10 mL of acetic acid (100), mix, and filter the solution.

12) Choline bitartrate

Accurately weigh sample equivalent to about 50 mg of choline bitartrate ($C_9H_{19}NO_7$), dissolve it in water to make exactly 100 mL. Use the resulting solution as the test solution. Separately, accurately weigh about 50 mg of choline bitartrate RS and add water to make it up to exactly 100 mL. Use the resulting solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 50 μL each of the test and standard solutions under the following conditions.

Amount (mg) of choline bitartrate ($C_9H_{19}NO_7$)
= Amount (mg) of choline bitartrate RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: Differential refractometer

Column: A stainless steel column, about 4.6 mm in internal diameter and about 30 cm in length, filled with octadecylsilyl silica gel for liquid chromatography.

Mobile phase: Dissolve 250 mL of methanol and 1.0 g of sodium 1-octanesulfonate in water to make 1 L, add 10 mL of acetic acid (100), mix, and filter the solution.

13) Thiamine disulfide

Accurately weigh sample equivalent to about 20 mg of thiamine disulfide ($C_{24}H_{34}N_8O_4S_2$), dissolve it in 0.01 mol/L hydrochloric acid by shaking vigorously, and make exactly 100 mL. Use the resulting solution as the test solution. Separately, accurately weigh about 20 mg of thiamine disulfide RS and dissolve it in 0.01 mol/L hydrochloric acid to make exactly 100 mL. Use the resulting solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

Amount (mg) of thiamine disulfide ($C_{24}H_{34}N_8O_4S_2$)
= Amount (mg) of thiamine disulfide RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Dissolve 350 mL of methanol and 1 g of sodium 1-hexanesulfonate in water to make 1000 mL, add 10 mL of acetic acid (100), mix, and filter.

14) D-*α*-tocopherol, tocopheryl acetate, D-*α*-tocopheryl acetate, tocopherol calcium succinate, and D-*α*-tocopherol calcium succinate

Accurately weigh sample equivalent to about 100 IU of vitamin E ($C_{22}H_{50}O_2$), dissolve it in 30 mL of methanol by shaking three times, and filter it. Combine all the filtrate and dissolve it in methanol to make sure that the final concentration of vitamin E is 1 IU/mL, and use it as the test solution. Separately, accurately weigh about 100 IU of tocopherol RS, D-α-tocopherol RS, tocopheryl acetate RS, D-α-tocopheryl acetate RS, calcium tocopherol succinate RS, or calcium D-α-tocopherol succinate RS, and dissolve it in methanol to make exactly 100 mL. Use the resulting solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 100 μL each of the test and standard solutions under the following conditions.

Amount (IU) of tocopherol ($C_{22}H_{50}O_2$)
= Amount (IU) of tocopherol RS × $\frac{A_T}{A_S}$

Amount (IU) of D-α-tocopherol ($C_{22}H_{50}O_2$)
= Amount (IU) of D-α-tocopherol RS × $\frac{A_T}{A_S}$

Amount (IU) of tocopheryl acetate ($C_{31}H_{52}O_3$)
= Amount (IU) of tocopheryl acetate RS × $\frac{A_T}{A_S}$

Amount (IU) of D-α-tocopheryl acetate ($C_{31}H_{52}O_3$)
= Amount (IU) of D-α-tocopheryl acetate RS × $\frac{A_T}{A_S}$

Amount (IU) of tocopherol calcium succinate ($C_{66}HO_{106}CaO_{10}$)
= Amount (IU) of tocopherol calcium succinate RS × $\frac{A_T}{A_S}$

Amount (IU) of D-α-tocopherol calcium succinate
$C_{66}HO_{106}CaO_{10}$)
= Amount (IU) of D-α-tocopherol calcium succinate RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column with an internal diameter of about 8 mm and a length of 10 cm, filled with porous octadecylsilyl silica gel or ceramic particles for liquid chromatography with particle size ranging from 5 to 10 μm.

Mobile phase: Mixture of water and methanol (5: 95).

15) Calcium pantothenate, sodium pantothenate, and dexapanthenol

Accurately weigh sample equivalent to about 15 mg of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), sodium pantothenate

($C_9H_{16}NaNO_4$), or dexpanthenol ($C_9H_{19}NO_4$), add 25 mL of the mobile phase, and mix by shaking. After centrifuging, take the supernatant and use it as the test solution. Separately, accurately weigh about 15 mg of calcium pantothenate, sodium pantothenate, or dexpanthenol RS and dissolve it in the internal standard solution to make exactly 25 mL. Use the resulting solution as the standard solution. Measure the peak area ratio Q_T and Q_S of each solution to the peak area of the internal standard solution by performing the test directed under liquid chromatography with 10 μL each of the test and standard solutions under the following conditions.

Amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)
= Amount (mg) of calcium pantothenate RS × $\frac{Q_S}{Q_T}$

Amount (mg) of sodium pantothenate ($C_9H_{16}NaNO_4$)
= Amount (mg) of sodium pantothenate RS × $\frac{Q_S}{Q_T}$

Amount (mg) of dexapanthenol ($C_9H_{19}NO_4$)
= Amount (mg) of dexapanthenol RS × $\frac{Q_S}{Q_T}$

Internal standard solution—Dissolve 80 mg of parahydroxybenzoic acid in 5 mL of methanol, and add the mobile phase to make it up to exactly 1 L.

Operating conditions

Detector: UV spectrophotometer (wavelength: 210 nm).

Column: A stainless steel column with an internal diameter of about 3.9 mm and a length of 15 cm, filled with porous octadecylsilyl silica or ceramic particles for liquid chromatography with particle size ranging from 5 to 105 μm.

Mobile phase: Mixture of water and phosphoric acid (1000: 1).

16) Fursultiamine and fursultiamine hydrochloride

Accurately weigh sample equivalent to about 20 mg of fursultiamine ($C_{17}H_{26}N_4O_3S_2$) or fursultiamine hydrochloride ($C_{17}H_{26}N_4O_3S_2.HCl$), add 20 mL of water, shake to mix, and dissolve it in methanol to make exactly 100 mL. Pipette 10 mL of this solution, add methanol to make exactly 100 mL, and use it as the test solution. Separately, accurately weigh about 20 mg of fursultiamine or fursultiamine hydrochloride RS and dissolve it in methanol to make exactly 100 mL. Take 10 mL of this solution, add methanol to make it up to exactly 100 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

Amount (mg) of fursultiamine ($C_{17}H_{26}N_4O_3S_2$)
= Amount (mg) of fursultiamine RS × $\frac{A_T}{A_S}$

Amount (mg) of fursultiamine hydrochloride ($C_{17}H_{26}N_4O_3S_2.HCl$)
= Amount (mg) of fursultiamine hydrochloride RS × $\frac{A_T}{A_S}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column with an internal diameter of 4.6 mm and a length of 30 cm, filled with porous aminopropylsilyl silica gel for liquid chromatography with a particle size of 3 μm.

Mobile phase: Add 1 g of sodium 1-hexanesulfonate to 350 mL of methanol, dissolve it in water to make 1000 mL, shake to mix, and filter it.

17) Phytonadione (vitamin K_1)

Accurately weigh sample equivalent to about 100 IU of phytonadione ($C_{31}H_{46}O_2$), dissolve it in 20 mL of methanol by shaking three times, and filter it. Combine all the filtrate, evaporate the methanol under reduced pressure, and dissolve the residue in methanol to make sure that the final concentration of phytonadione is 20 μg/mL. Use the resulting solution as the test solution. Separately, accurately weigh about 20 mg of phytonadione RS and dissolve it in mobile phase to make exactly 100 mL. Take 10 mL of this solution, add methanol to make it up to exactly 100 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 10 μL each of the test and standard solutions under the following conditions.

Amount (mg) of phytonadione ($C_{31}H_{46}O_2$)
= Amount (mg) of phytonadione RS × $\frac{A_T}{A_S} \times \frac{1}{200}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column with an internal, diameter of about 8 mm and a length of 10 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Mixture of methanol and water (95: 5).

18) Hydroxocobalamin, hydroxocobalamin hydrochloride, and hydroxocobalamin acetate

Accurately weigh sample, equivalent to about 1 mg of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{19}P$), hydroxocobalamin hydrochloride ($C_{62}H_{89}CoN_{13}O_{15}P.HCl$), or hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{18}P.C_2H_4O_2$), and dissolve it in water to make exactly 500 mL. Use the resulting solution as the test solution. Separately, accurately weigh about 10 mg of hydroxocobalamin, hydroxocobalamin hydrochloride, or hydroxocobalamin acetate RS and dissolve it in water to make 500 mL. Pipette 5 mL of the resulting solution, add water to make it up to exactly 50 mL, and use this solution as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

Amount (mg) of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{19}P$)
= Amount (mg) of hydroxocobalamin RS × $\frac{A_T}{A_S} \times \frac{1}{10}$

Amount (mg) of hydroxocobalamin hydrochloride ($C_{62}H_{89}CoN_{13}O_{15}P.HCl$)
= Amount (mg) of hydroxocobalamin hydrochloride RS × $\frac{A_T}{A_S} \times \frac{1}{10}$

Amount (mg) of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{18}P.C_2H_4O_2$)
= Amount (mg) of hydroxocobalamin acetate RS × $\frac{A_T}{A_S} \times \frac{1}{10}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 361 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile phase: Dissolve 590 mL of methanol and 1 g of sodium 1-octanesulfonate in water to make 1000 mL, add 10 mL of acetic acid (100), shake to mix, and filter.

19) Orotic acid

Accurately weigh sample equivalent to about 15 mg of orotic acid ($C_5H_4N_2O.H_2O$) and add 1 mL of sodium hydroxide TS. Add sodium bicarbonate solution (1 in 1000) and dissolve to make exactly 500 mL, and use it as the test solution. Separately, accurately weigh about 15 mg of orotic acid RS and add 1 mL of sodium hydroxide TS. Then, add sodium bicarbonate solution (1 in 1000) to make exactly 500 mL. Use this solution as the standard solution. Measure the peak areas A_S and A_T of each solution by performing the test directed under liquid chromatography with the standard and test solutions under the following conditions.

$$\text{Amount (mg) of orotic acid } (C_5H_4N_2O.H_2O) = \text{Amount (mg) of orotic acid RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 270 nm).

Column: A stainless steel column with an internal diameter of about 3.9 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 10 μm.

Mobile phase: 20% methanol

Column temperature: 40 ℃

20) DL-Carnitine hydrochloride

Accurately weigh sample equivalent to about 0.1 g of DL-carnitine hydrochloride ($C_7H_{16}O_3.HCl$), and dissolve it in water to make exactly 100 mL. Use the resulting solution as the test solution. Separately, weigh about 0.1 g of DL-carnitine hydrochloride RS and dissolve it in water to make exactly 100 mL. Use the resulting solution as the standard solution. Measure the peak areas A_S and A_T of each solution by performing the test directed under the liquid chromatography with the standard and test solutions under the following conditions.

$$\text{Amount (mg) of DL-carnitine hydrochloride } (C_7H_{16}O_3.HCl) = \text{Amount (mg) of DL-carnitine hydrochloride RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 214 nm).

Column: A stainless steel column with an internal diameter of about 3.9 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 10 μm.

Mobile: 0.061 mol/L phosphoric acid solution

21) Riboflavin butyrate

Accurately weigh sample equivalent to about 5 mg of riboflavin butyrate ($C_{33}H_{44}N_4O_{10}$), and dissolve it in methanol to make exactly 100 mL. Use the resulting solution as the test solution. Separately, weigh about 50 mg of riboflavin butyrate RS accurately and dissolve it in methanol to make exactly 100 mL. Take 10.0 mL of the resulting solution, add methanol to make it up to 100 mL, and use it as the standard solution. Measure the peak areas A_T and A_S of each solution by performing the test directed under the liquid chromatography with 20 μL each of the test and standard solutions under the following conditions.

$$\text{Amount (mg) of riboflavin butyrate } (C_{33}H_{44}N_4O_{10}) = \text{Amount (mg) of riboflavin butyrate RS} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: UV spectrophotometer (wavelength: 269 nm).

Column: A stainless steel column with an internal diameter of about 4.6 mm and a length of 25 cm, filled with octadecylsilyl silica gel for liquid chromatography with a particle size of 5 μm.

Mobile: Mixture of 1% phosphoric acid and methanol (20: 80).

Test Solutions

1) Antimony pentachloride TS: Dissolve 2 mg of antimony pentachloride in 8 mL of chloroform.

2) 2,6-dichloroquinone chloroimide TS: Dissolve 1 g of 2, 6-dichloroquinone chloroimide in 100 mL of methanol.

3) *o*-Tolidine/potassium iodide TS: Dissolve 0.16 g of *o*-tolidine in 30 mL of acetic acid (100) and add water to make it up to 500 mL. To this solution, add 1 g of potassium iodide and dissolve it.

Antimicrobial Preservatives Analysis
보존제시험법

1. Specifications

A. Identification

Each preservative listed on the label must be identified.

B. Content

For liquid preparations for oral administration (including dry syrup), the content should be NMT the labeled amount, and for all other preparations, the content should be between 80.0% and 120.0% of the labeled amount. If necessary, the acceptance criteria can be specified in an individual monograph.

2. Test method

A. Identification

The retention time of the major peak of the test solution corresponds to that of the standard solution, as obtained in the Assay.

B. Assay

The final concentrations of the test and standard solutions can be adjusted to the same in the range of 5 - 50 μg/mL for simultaneous analysis of the preservative components.

1) Liquid chromatography

a) Parahydroxybenzoic acid esters and their salts

Test solution: Weigh accurately an appropriate amount of the test preparation according to the labeled preservative content, add methanol to make 10 μg/mL for each preservative, and filter. For non-liquid preparations such as soft capsules, add warm water, shake to completely dissolve the soft capsule, and adjust the concentration to be equal to that of the standard solution by adding methanol, and filter. If a large amount of sweeteners (sugars) such as syrups are contained in the preparations, remove as much of the sugars as possible by solid phase extraction or other appropriate methods, and filter.

Standard solution: Weigh accurately an appropriate amount of the reference standard for each preservative, and dissolve it in methanol to make a final concentration of 10 μg/mL.

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operation conditions and calculate the amount of the preservative using the peak areas of the preservative from the test (A_T) and standard solution (A_S).

Amount of the preservative (mg)
= Concentration of the standard solution (mg/mL) × dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of acetonitrile, water and acetic anhydride (55 : 44 : 1).

b) Benzoic acid and its salts, dehydroacetate and its salts

Test solution: prepare as described in A).

Standard solution: prepare as described in A).

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of preservative using peak areas of the preservative from the sample (A_T)and standard (A_s) solution.

Amount of the preservative (mg)
= concentration of the standard solution (mg/mL)×dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in interna diameter and 15 to 30 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of 0.01 mol/L ammonium monohydrogen phosphate in methanol, tetrahydrofuran and water (225 : 60 : 715), adjusted to pH 3.4 with phosphoric acid.

c) Sorbic acid and its salts

Test solution: prepare as described in A).

Standard solution: prepare as described in A).

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of the preservative using the peak areas of the preservative from the sample (A_T) and standard (A_s) solution.

Amount of the preservative (mg)
= Concentration of the standard solution (mg/mL)×dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of 0.01 mol/L ammonium dihydrogen phosphate in acetonitrile and water (35 : 65), adjusted to pH 2.5 with phosphoric acid.

d) Benzyl alcohol

Test solution: prepare as described in A).

Standard solution: prepare as described in A).

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of benzyl alcohol using peak areas of benzyl alcohol from the sample (A_T) and standard (A_s) solution.

Amount of benzyl alcohol (mg)
= Concentration of the standard solution (mg/mL)×dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of 0.005 mol/L 1-sodium 1-octanesulfonate in methanol, water and acetic anhydride (20:79:1).

e) Chlorobutanol

Test solution: prepare as described in A).

Standard solution: prepare as described in A).

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of chlorobutanol using peak areas of chlorobutanol from the test (A_T) and standard (A_s) solution.

Amount of chlorobutanol (mg)
= Concentration of the standard solution (mg/mL) ×dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter mm and 15 to 30 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 to 10 μm.

Mobile phase: A mixture of 0.005 mol/L 1-sodium 1-octanesulfonate in acetonitrile and water (40:60), adjusted to pH 3.0 with phosphoric acid.

f) Phenol

Test solution: prepare as described in A).

Standard solution: prepare as described in A).

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of phenol using the peak areas of phenol from the test (A_T) and standard (A_s) solution.

Amount of phenol (mg)
= Concentration of the standard solution (mg/mL) ×dilution factor of the test solution (mL)× $\frac{A_T}{A_S}$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 15 to 30 cm in length, packed with octadecylsilyl

silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of methanol and water (70: 30).

g) Benzalkonium chloride

Test solution: prepare as described in A) except using mobile phase instead of methanol as a solvent.

Standard solution: prepare as described in A) except using mobile phase instead of methanol as a solvent.

Analysis: Perform the test according to the Liquid Chromatography with the test and standard solutions under the following operating conditions and calculate the amount of benzalkonium chloride using the sum of peak areas of benzalkonium chloride from the test (A_T) and standard (A_S) solution.

$$\text{Amount of benzalkonium chloride (mg)} = \text{Concentration of the standard solution (mg/mL)} \times \text{dilution factor of the test solution (mL)} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 254 nm).

Column: A stainless steel column about 4 mm in internal diameter and 15 to 30 cm in length, packed with nitrated silica gel for liquid chromatography (particle diameter of 5 to 10 μm).

Mobile phase: A mixture of acetonitrile and 1% sodium monohydrogen phosphate (50:50), adjusted to pH 5.2 with phosphoric acid.

h) Cresol, chlorocresol, and benzethonium chloride

Test solution: Weigh accurately an appropriate amount of the test preparation according to the labeled preservative content, add methanol to make a final concentration of 100 μg/mL for each preservative, and filter.

Standard solution: Weigh accurately an appropriate amount of the reference standard for each preservative and dissolve it in methanol to make a final concentration of 100 μg/mL.

Analysis: Perform the test according to the Liquid Chromatography with 20 μL of the test and standard solutions under the following operating conditions and calculate the amount of preservative using peak areas of the preservative from the test (A_T) and standard (A_S) solution.

$$\text{Amount (mg) of the preservative} = \text{Concentration (mg/mL) of the standard solution} \times \text{dilution factor of the test solution (mL)} \times \frac{A_T}{A_S}$$

Operating conditions

Detector: Ultraviolet absorption photometer (measurement wavelength: 275 nm).

Column: A stainless steel column about 4.6 mm in internal diameter of about 4.6 mm and 25 cm in length, packed with octadecylsilyl silica gel for liquid chromatography (particle diameter of 5 μm).

Column temperature: Constant temperature around 40 ℃.

Mobile phase A: Put distilled water in a 1-L volumetric flask, add 631 mg of ammonium formate, then add distilled water up to the gauge line, and adjust the pH to 3.3 with formic acid.

Mobile phase B: Methanol

Time (min)	Mobile Phase A (Vol%)	Mobile Phase B (Vol%)	Elution
0.0 – 5.0	40	60	Equilibrium
5.0 – 10.0	40 → 30	60 → 70	Linear gradient
10.0 – 20.0	30 → 20	70 → 80	Linear gradient

Flow rate: 1.0 mL/min

System suitability

System repeatability: NMT 1.0% of the peak areas for six consecutive injections of 20 μL standard solution.

2) Gas chromatography

a) Para-hydroxybenzoic acid esters and their salts, benzyl alcohol, chlorobutanol, and phenol: Perform the test according to the Antimicrobial Effectiveness Testing in US Pharmacopoeia.

b) Benzoic acid and its salts, sorbic acid and its salts, dehydroacetate and its salt: Weigh accurately 5 - 10 mg of the preservative according to the labeled amount. If necessary, add 50 mL of water and heat to dissolve. Add 10 g of sodium chloride and 5 mL of dilute hydrochloric acid, and extract 3 times with 40 mL, 30 mL, and 30 mL of ether respectively. Combine the ether extract, wash it with a small amount of water, and collect the ether layer separately. Dry the ether layer with anhydrous sodium sulfate and then evaporate the ether. Dissolve the residue in the internal standard solution to make 10 mL and use this as the test solution. Separately, weigh accurately a certain amount of reference standard of each preservative and make in the same procedure as the test solution and use this solution as the standard solution. Perform the test with the test solution and standard solution as direction under the Gas Chromatography according to the following conditions and determine the ratio of the peak area of each preservative to that of the internal standard, Q_T and Q_S

$$\text{Amount of the preservative (mg)} = \text{Concentration of the standard solution (mg/mL)} \times \text{dilution factor of the test solution (mL)} \times \frac{Q_T}{Q_S}$$

Internal standard solution: Acetone solution of acetanilide (1 in 1,000).

Operating conditions

Detector: Flame ionization detector

Column: ① A column about 3 mm in internal diameter and about 2 m in length, packed with diatomaceous earth of 60 to 80 mesh coated with 2 ~ 5% of diethylene glycol succinate and 1% of phosphoric acid for gas chromatography.

② A column about 3 mm in internal diameter and about 2 m in length, packed with diatomaceous earth of 60 to 80 mesh coated with 10% neopentyl glycol succinate and 1% phosphoric acid for gas chromatography.

Injection port temperature: Constant temperature of 210 - 230 ℃

Column temperature: Constant temperature of 140 - 200 ℃

Detector temperature: Constant temperature of 230 - 250 ℃

Carrier gas: Nitrogen

Flow rate: 60 mL/min

Arsenic
비소시험법

The Arsenic is a limit test of arsenic contained in drugs. The limit is expressed as the amount of arsenic trioxide (As_2O_3). In the monograph, the limit of arsenic (As_2O_3) is expressed in ppm in parentheses.

Apparatus

Use the apparatus shown in the figure. Fill a vent pipe B with glass wool F with a height of about 30 mm, moisten it evenly with a mixture of lead acetate TS and water at the same ratio, and aspirate gently at the bottom to remove the excess solution. Vertically insert it in the center of the rubber stopper H, lower the small hole E at the bottom of B below the rubber stopper, and insert it in the generating bottle A. On the top end of B, insert a rubber stopper I, which is vertically fixed on the glass column C. Make sure that the lower end of the vent pipe of C is on the same plane as the lower end of the rubber stopper I.

Preparation of the test solution

Unless otherwise specified, prepare it by the following method.

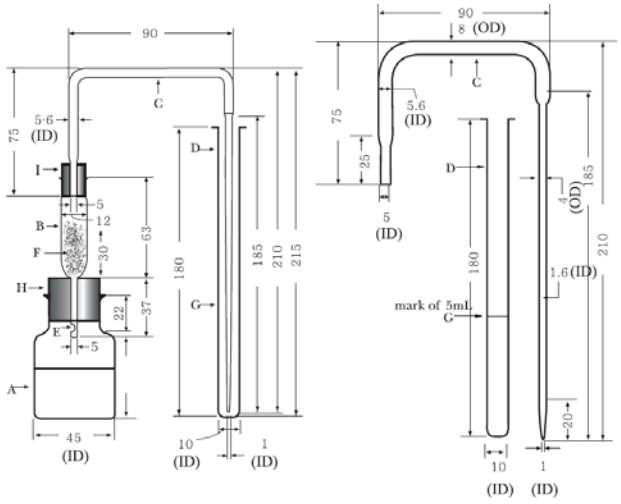
Method 1: Weigh the amount of sample specified in the monograph, add 5 mL of water, dissolve by heating, if necessary, and use it as the test solution.

Method 2: Weigh the amount of sample specified in the monograph and add 5 mL of water and 1 mL of sulfuric acid. However, do not add sulfuric acid in the case of a mineral acid. Add 10 mL of sulfurous acid water, transfer it to a small beaker, and heat on a water bath until it evaporates and the volume becomes about 2 mL. Add water to reach 5 mL and use it as the test solution.

Method 3: Weigh the amount of sample specified in the monograph and place it into a platinum, quartz, or porcelain crucible. Add 10 mL of ethanol solution of magnesium nitrate (1 in 50), ignite in ethanol to burn it, and slowly incinerate by heating. If a carbonized substance remains, moisten it with a small amount of nitric acid, and incinerate it by ignition. After cooling, add 3 mL of hydrochloric acid to the residue, and dissolve by heating on a water bath. Use it as the test solution.

Method 4: Weigh the amount of sample specified in the monograph and place it into a platinum, quartz, or porcelain crucible. Add 10 mL of ethanol solution of magnesium nitrate (1 in 10) to it, ignite in ethanol to burn, and slowly incinerate by heating. If a carbonized substance remains, moisten it with a small amount of nitric acid, heat slowly, and incinerate by ignition. After cooling, add 3 mL of hydrochloric acid to the residue, and heat on a water bath to dissolve. Use it as the test solution.

Method 5: Weigh the amount of sample specified in the monograph, add 10 mL of N, N-dimethylformamide, dissolve by heating, and use it as the test solution.



* The figures are in mm.

Figure

A: Generating bottle (volume to the shoulder part is about 70 mL)
B: Vent pipe
C: Glass tube (internal diameter of 5.6 mm, extend the part to be put in the absorption pipe until the internal diameter becomes 1 mm)
D: Absorption pipe (internal diameter of 10 mm)
E: Small hole
F: Glass wool (about 0.2 g)
G: 5 mL of gauge line
H and I: Rubber stopper
ID: Internal diameter
OD: Outer diameter

Test solution

1) Arsenic hydrogen absorbing solution

Dissolve 0.05 g of silver *N*, *N*-diethyldithiocarbamate in pyridine to make 100 mL. Store the solution in a bottle with a stopper in a cold room shaded from the light.

2) Arsenic standard stock solution

Pulverize arsenic trioxide into a very fine powder, dry at 105 ℃ for 4 hours, weigh accurately 0.100 g of it, and dissolve it in 5 mL of sodium hydroxide solution (1 in 5). Add dilute sulfuric acid to the solution to neutralize, and add 10 mL of dilute sulfuric acid again. Add freshly boiled and cooled water to make exactly 1000 mL.

3) Arsenic standard solution

Accurately take 10 mL of the arsenic standard stock solution and add 10 mL of dilute sulfuric acid. Add newly boiled and cooled water to make exactly 1000 mL. 1 mL of the solution contains 1 μg of arsenic trioxide (As_2O_3). Prepare the solution before use and store it in a bottle with a stopper.

Procedure

Unless otherwise specified, the test is performed according to the following methods. Prepare the standard color preparation at the same time. Take the test solution from in the generating bottle A, cleanse with a small quantity of water, if necessary, and add 1 drop of methyl orange TS. Neutralize with ammonia TS, ammonia water (28), or dilute hydrochloric acid. Add 5 mL of dilute hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acid tin (II) chloride TS again and allow to stand at ordinary temperature for 10 minutes. Add water to the solution to make 40 mL, add 2 g of zinc for arsenic assay, and insert the rubber stopper H connecting B and C on the generating bottle A. The end of tube C should be inserted to reach the bottom of the absorber tube D,

where 5 mL of arsenic hydrogen absorbing solution was filled in advance. Next, place the generating bottle A in 25 ℃ of water until submerged up to the shoulder and allow to stand for 1 hour. Remove the absorber tube, add 5 mL of pyridine, if necessary, and observe the color of the absorbing solution. The color is no darker than the standard color.

Preparation of standard color

Accurately add 2 mL of arsenic standard solution in the generating bottle A, add 5 mL of dilute hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic stannous chloride TS, and allow to stand at ordinary temperature for 10 minutes. The color of the absorbing solution obtained following the above procedures is used as the standard color. This color corresponds to 2 μg of arsenic trioxide (As_2O_3).

Cautions

The apparatus used in the test, reagent, and TS should contain no or little arsenic. If necessary, perform a blank test.

Atomic Absorption Spectroscopy
원자흡광광도법

The Atomic Absorption Spectroscopy is a method that measures the amount (concentration) of an analyte element in a sample by using a phenomenon in which atoms in the ground state absorb light of a specific wavelength when the light passes through an atomic vapor layer. When testing according to the Atomic Absorption Spectroscopy, Inductively Coupled Plasma Spectrochemistry can be used in this test if proven that it does not affect the results.

Apparatus

It usually consists of a light source unit, a sample atomizer unit, a spectrometer unit, a photometric unit, and a recording unit. Some instruments have a background correction unit. Hollow cathode lamps, discharge lamps, etc., are used as a light source unit. The sample atomizer unit uses a flame method, an electrothermal method, or a cold vapor method. The cold vapor atomization method includes a chemical reduction-vaporization method and a thermal vaporization method. The flame atomization consists of a burner and gas flow controller, the electrothermal atomization consists of an electric furnace and power source, and the cold vapor atomization consists of mercury generation units such as a chemical reducing vaporizer, heating vaporizer, etc., and absorption cells. In the spectroscopic unit, a diffraction grating or interference filter is used. The photometric unit consists of a detector, a signal processing system, etc. The display recording unit includes a screen and a recording device. The background correction unit is used to correct the background line, which includes the continuous spectrum light source method, the Zeeman method, the non-resonance spectrum method, and the self-inversion method. Special apparatus such as hydride generator and heating absorption cells can be used for the analysis of selenium, etc. The hydride generator is available as a batch and continuous flow type, and the heating-absorption cell is for heating by flame or electric furnace.

Procedure

Unless otherwise specified, proceed by one of the following methods.

1) Flame method

Insert the specified light source lamp and turn it on by supplying power to the photometric unit. Tune in the spectrometer to the analytical wavelength specified in the monograph, and set the appropriate current value and slit width. Next, ignite the mixed gases with the specified oxidizing gas and combustible gas, adjust the gas flow rate and pressure, and spray the solvent into the flame to set the zero point. Spray the test solution prepared according to the specified method into the flame and measure its absorbance.

2) Electrothermal method

Insert the specified light source lamp and turn it on by supplying power to the photometric unit. Tune in the spectrometer to the analytical wavelength specified in the monograph, and set the appropriate current value and slit width. Inject a certain amount of the test solution prepared according to the specified method into an electric furnace (heating element) and let an appropriate amount of gas flow. Adjust the temperature, time, and heating method appropriately, and measure the absorbance by drying, ashing, and atomizing.

3) Cold vapor method

Insert the specified low-pressure mercury lamp and turn it on by supplying power to the photometric unit. Tune in the spectrometer to the analytical wavelength specified in the monograph, and set the appropriate current value and slit width. In the chemical reducing vaporization method, take the test solution prepared according to the specified method into a sealed container, add a suitable reducing agent, reduce and vaporize it until it becomes an elemental form. In addition, in the heating vaporization method, the sample is heated and vaporized. Measure the absorbance of the resulting atomic vapor this way.

Assay

In general, proceed by one of the following methods. In the determination, interferences and background lines should be considered.

1) Calibration curve method

Prepare more than three standard solutions with different concentrations, measure the absorbance of each standard solution, and prepare a calibration curve from the obtained measurements. After measuring the absorbance of the test solution so that it falls within the measurable concentration range, determine the amount (concentration) of the analyte element from the calibration curve.

2) Standard addition method

Take more than three test solutions of the same amount, add the standard solution of the analyte element to each of them to achieve a stepwise concentration, constant. and add the solvent again to obtain a certain volume. Measure the absorbance of each solution and prepare a calibration curve with the amount of the added standard analyte element on the horizontal axis and the absorbance on the vertical axis. Extend the regression line obtained from the calibration curve to calculate the amount (concentration) of the analyte element at a distance between the intersection with the horizontal axis and the origin. However, this method is applicable only when the calibration curve in 1) is a straight line passing through the origin.

3) Internal standard method

Prepare a standard solution by keeping the amount of the internal standard element constant and adding known amounts of

the standard sample element to have stepwise concentrations. With each standard solution, measure the absorbance of the standard analyte element and the internal standard element under the same analytical conditions at the wavelength of the analytical line of each element. Then, determine the ratio between the absorbance of the standard analyte element and the internal standard element. Prepare a calibration curve with the amount (concentration) of the standard analyte element on the horizontal axis and the absorbance ratio on the vertical axis. When preparing the test solution, add the same amount of the internal standard element as for the standard solution in advance. Then, determine the ratio between the absorbance of the analyte element and the internal standard element obtained under the same analytical conditions as those used to prepare the calibration curve to determine the amount (concentration) of the analyte element from the calibration curve.

Note: Reagents, test solutions, and gases used in this test should not interfere with any measurement process.

Bacterial Endotoxins
엔도톡신시험법

The Bacterial Endotoxins is performed to detect and quantify endotoxins from gram-negative bacteria using amoebocyte lysate made from blood cell extracted from the *Limulus polyphemus* or *Tachypleus tridentatus*. This test includes a gel-clot method, in which gel formation of lysate TS by the action of endotoxin is used as an indicator, and a photometric measurement method, in which optical change is used as an indicator. The photometric measurement method includes a turbidimetric assay which is based on changes in turbidity during the gelation process of the lysate TS, and a chromogenic assay, which is based on the color development during hydrolysis of a synthetic substrate as an indicator.

The bacterial endotoxins test is performed using gel-clot method, turbidimetric or chromogenic assay. However, if there is any doubt regarding the result, the final decision shall be based on the gel-clot method, unless otherwise specified.

The test should be performed in such a way as to avoid endotoxin contamination.

Apparatus

All glassware and other heat resistant apparatus used in the test should be dry-heated through a validated process. The typical minimum time and temperature setting is 30 minutes at 250 °C. When using plastic apparatus, such as microplates and micro pipette tips, it is essential to ensure that endotoxins are not detected and they do not interfere with the test.

Preparation of the standard endotoxin stock solution

An endotoxin standard stock solution is prepared by dissolving an endotoxin reference standard in the Korean Pharmacopoeia (KP) that has been calibrated to the current WHO international standards in water for bacterial endotoxins test (BET). The endotoxin unit is expressed as EU. One EU is equal to one international unit (IU) of endotoxin.

Preparation of the endotoxin standard solution

After vigorously mixing the standard endotoxin stock solution, prepare the endotoxin standard solution by diluting it in water for the BET. Use the prepared endotoxin standard solution as soon as possible to prevent endotoxin adsorption into the container.

Preparation of the test solution

Unless otherwise specified, prepare the test solution by dissolving or diluting drugs using water for BET. Depending on the drug, the test solution can be prepared by adding necessary substances to water for BET. Sample solutions for containers for medicines should be prepared according to other specified procedures. Usually, the pH of the test solution should be in the range of 6.0 to 8.0. The pH may be adjusted using an acid, a base, or a suitable buffering solution. If the pH of the test solution mixed with the lysate TS exceeds the pH indicated on the lysate TS, pH adjustment may be required. TS or solutions used for pH adjustment should be prepared using water for BET and stored in a container where endotoxin is not detected. TS or solutions should be validated as free of detectable endotoxin and other reaction interfering factors.

Determination of the maximum valid dilution

The maximum valid dilution (MVD) is the maximum allowable dilution factor of the test solution if a factor interfering with the reaction is present in the test solution and thus is to be removed by dilution.

Determine the MVD using the following equation:

$$\text{Maximum Valid Dilution} = \frac{\text{Endotoxin limit} \times \text{Concentration of test solution}}{\lambda}$$

Endotoxin limit The endotoxin limit for injections is defined based on the dose and is equal to K/M. K represents the minimum pyrogenic dose of endotoxin per kg of body weight (EU/kg), and is determined as shown in the following table, depending on the administration route. The K values for the intravenous route are applicable to drugs that are administered by any route not specified in the table.

Intended route of administration	*K* (EU/kg)
Intravenous	5.0
Intravenous, for radiopharmaceuticals	2.5
Intrathecal	0.2

M is the maximum amount of injections administered per kilogram of body weight. If the injection is administered several times or regularly, M is the maximum total dose administered in a one-hour period. The unit of M is expressed in mL/kg when the dose is based on the volume of the drug, in mg/kg or mEq/kg when the dose is based on the mass of the drug, and in unit/kg when the dose is based on the biological unit of the drug. For preparations to be administered by mass or by units, the endotoxin limit should be established based on the labeled amount of the drug.

When calculating the maximum dose per 1 kg of adult body weight, use 60 kg as the average body weight of an adult. If the pediatric dose per 1 kg of body weight is higher than the adult dose, the endotoxin limit should be established based on the pediatric dose.

Concentration of the test solution The unit is mg/mL when the endotoxin limit is defined per weight (EU/mg), mEq/mL when it is defined per equivalent (EU/mEq), unit/mL when it is defined per biological unit (EU/unit), and mL/mL when it is defined per dose (EU/mL).

λ The labeled lysate sensitivity (EU/mL) in the gel-clot

technique or the lowest endotoxin concentration used (EU/mL) in the standard calibration curve of the turbidimetric or chromogenic assays.

Gel-clot Technique

The gel-clot technique allows for the detection and quantification of endotoxins and is based on the clotting of the lysate in the presence of endotoxins. To ensure both precision and validity of the test, perform the test to confirm the labeled lysate sensitivity and interfering factors as described in Preparatory Testing.

1) Preparatory testing

a) Confirmation of the labeled lysate sensitivity

The labeled sensitivity of the lysate reagent is defined as the lowest endotoxin concentration required to induce clotting in the lysate TS under the specified conditions for the use of lysate reagent. Confirm the labeled sensitivity λ, of each lot of the lysate reagent before use in the test. Confirmation of lysate sensitivity is performed when test conditions change, which could affect the result of the test.

Perform the test through the following procedures.

Prepare endotoxin standard solutions of 4 concentrations equivalent to 2 λ, 1 λ, 0.5 λ and 0.25 λ by diluting the standard endotoxin stock solution with water for BET. Prepare the lysate TS by dissolving the lysate reagent with water for BET or a suitable buffer.

Mix a volume of the lysate TS and an equal volume of endotoxin standard solutions (usually, 0.10 mL aliquots) in each test. When using reagents for a single test containing lyophilized lysate, add endotoxin standard solutions directly to the container to dissolve lysate reagents. Allow the test tube or container to stand at 37 ± 1 °C for 60 ± 2 min avoiding vibration, and invert it through approximately 180° in "one smooth motion" to observe the contents. If a firm gel has formed that remains in place upon inversion, record the result as positive. The test result is negative if either a firm gel is not formed, or if a fragile gel has formed but flows out upon inversion.

Making the endotoxin standard solutions of the four concentrations one set, test four replicates of the set.

The test is considered valid when the endotoxin standard solution at a concentration of 0.25 λ shows a negative result in all replicate tests. If the test is not valid, repeat the test after verifying the test conditions.

The endpoint is the minimum endotoxin concentration in each replicate that clots the lysate. Calculate the geometric mean endpoint concentration using the following formula:

$$\text{Geometric mean endpoint concentration} = \text{antilog}\,(\sum e/f)$$

$\sum e$: Sum of the log endpoint concentrations of the dilution series used.

f: Number of replicates

If the geometric mean endpoint concentration obtained is in the range of 0.5 to 2.0 λ, the labeled lysate sensitivity is confirmed, and is used for the following test.

b) Test for interfering factors

This test is performed to check for the presence of enhancing or inhibiting factors for the reaction in test solutions. Prepare solutions A, B, C, and D as shown in Table 1. Perform the test four times with solutions A and B and twice with solutions C and D. Reaction temperature, time, and determination of gel formation follow the procedure in 1) Preparatory testing a) Confirmation of the labeled lysate sensitivity. The geometric mean endpoint concentrations of solutions B and C are determined using the formula described in 1) Preparatory testing a) Confirmation of the labeled lysate sensitivity. The test for interfering factors must be repeated if changes are made to the experimental conditions that could affect the result of the test. The test is considered valid if the test results of solutions A and D are negative and the result of solution C confirms the labeled lysate sensitivity. If the geometric mean endpoint concentration of solution B is NLT 0.5 and not greater than 2.0 λ, the test solution does not contain interfering factors and complies with the test for interfering factors. Otherwise, the test solution interferes with the test. If the test solution interferes with the test at a dilution below the MVD, repeat the test for interfering factors with a larger dilution that does not exceed the MVD. The use of a more sensitive lysate permits a greater dilution of the test solution to be examined. Furthermore, interfering factors in the test solution or diluted test solution may be eliminated by suitable treatments, such as filtration, neutralization, dialysis or heat treatment. To determine if the selected treatment effectively removes the interfering factors without losing endotoxins, perform the test described above with the test solution to which the standard endotoxin has been added and then subjected to the selected treatment.

Table 1

Solution	Concentration of added endotoxin in each solution / Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A[1)]	0 / Test solution	-	-	-	4
B[2)]	2 λ / Test solution	Test solution	1 2 4 8	2 λ 1 λ 0.5 λ 0.25 λ	4
C[3)]	2 λ / Water for BET	Water for BET	1 2 4 8	2 λ 1 λ 0.5 λ 0.25 λ	2
D[4)]	0 / Water for BET	-	-	-	2

1) Negative control. Test solution only
2) Test solutions added with standard endotoxin for testing interfering factors
3) Endotoxin standard solutions for confirmation of the labeled lysate sensitivity
4) Negative control, water for BET only

2) Limit test

This test is a method used to determine whether a sample contains endotoxin in excess of the endotoxin limit specified in the monograph by gel formation according to the labeled sensitivity of the lysate reagent.

a) Procedure

Prepare the solutions A, B, C, and D according to Table 2. Consider these solutions as one set, and test two replicates of the set. When preparing the test solutions of solutions A and B, use a suitable solution that complies with 1) Preparatory testing b) Test for interfering factors. Reaction temperature, time, and determination of gel formation follow the procedure in 1) Preparatory testing a) Confirmation of the labeled lysate sensitivity.

Table 2

Solution	Endotoxin Concentration/ Solution to which endotoxin is added	Number of replicates
A[1)]	0 / Test solution	2
B[2)]	2 λ / Test solution	2

C[3)]	2 λ / Water for BET	2
D[4)]	0 / Water for BET	2

1) Test solution for the limit test. The test solution may be diluted to not greater than the MVD.
2) Positive control. Solution to which the standard endotoxin is added so that the final concentration is 2 λ in the test solution diluted with the same dilution as solution A.
3) Positive control. Endotoxin standard solution at a concentration of 2 λ.
4) Negative control. Water for BET only

b) Interpretation

The test is considered valid when both replicates of solution B and C are positive and those of solution D are negative. When a negative result is found for both replicates of solution A, the sample complies with the test. When a positive result is found for both replicates of solution A, the sample does not comply with the test. Repeat the test in duplicate when the test results are positive for one test but negative for the other one. The sample meets the endotoxin limit requirement of the test when a negative result is found for both replicates of solution A in the repeat test. The sample does not meet the endotoxin limit requirements if all or one of the two repeated tests comes out positive.

However, if the result is positive, and the dilution factor of the test solution is less than the MVD, the test may be repeated by dilution with the MVD or a dilution factor that does not exceed the MVD.

3) Quantitative test

The test measures endotoxin concentration in the test solution by determining an endpoint through gel formation.

a) Procedure

Prepare solutions A, B, C, and D as shown in Table 3. These four solutions are considered as one set; perform the test with two replicates of the set. When preparing test solutions A and B, use a suitable solution that complies with 1) Preparatory testing b) Test for interfering factors. The assay procedure follows the outline provided in 1) Preparatory testing a) Confirmation of the labeled lysate sensitivity.

Table 3

Solution	Concentration of added endotoxin in each solution / Solution to which endotoxin is added	Diluent	Dilution factor*	Endotoxin concentration	Number of replicates
A[1)]	0 / Test solution	Water for BET	1 2 4 8	- - - -	2
B[2)]	2 λ / Test solution	-	1	2 λ	2
C[3)]	2 λ / Water for BET	Water for BET	1 2 4 8	2 λ 1 λ 0.5 λ 0.25 λ	2
D[4)]	0 / Water for BET	-	-	-	2

1) Test solution for the assay. The serial dilution factor can be changed accordingly within the range that does not exceed the maximum valid dilution.
2) Positive control. Solution to which the standard endotoxin is added so that the final concentration is 2 λ to the test solution diluted by the same dilution factor as the maximum dilution factor of solution A.
3) Endotoxin standard solutions for confirmation of the labeled lysate sensitivity.
4) Negative control. Water for BET only

b) Calculation and interpretation

The test is valid when the following three conditions are met: (a) both replicates of solution D (negative control) are negative, (b) both replicates of solution B (positive product control) are positive, and (c) the geometric mean end-point concentration of solution C is in the range of 0.5 to 2.0 λ. The endpoint is defined as the maximum dilution showing a positive result in the dilution series of solution A, and the endotoxin concentration of the test solution is calculated by multiplying the endpoint dilution factor by λ. When none of the dilution series of solution A shows positive results, the concentration of endotoxin in the test solution is less than the value λ multiplied by the minimum dilution factor of solution A. If all dilutions are positive in the dilution series of solution A, the endotoxin concentration of the test solution is reported as equal to or greater than the largest dilution factor of solution A multiplied by λ. Calculate the endotoxin concentration (in EU/mL, EU/mg, EU/mEq, or EU/unit) of the sample, based on the endotoxin concentration of the test solution. The sample complies with the bacterial endotoxins test if the endotoxin concentration of the sample in both replicates meets the requirement for the endotoxin limit (in EU/mL, EU/mg, EU/mEq, or EU/unit) specified in the monograph.

Photometric techniques

1) Turbidimetric assay

In this technique, the endotoxin concentration of the test solution is determined by measuring the change in turbidity associated with the gelation of the lysate TS. Based on the test principle used, this technique can be classified as either the endpoint-turbidimetric method or the kinetic-turbidimetric method. The endpoint-turbidimetric method is based on the dose-response relationship between the endotoxin concentration and the turbidity of the reaction mixture after a certain time. The kinetic-turbidimetric method is used to measure either the time needed for the reaction mixture to reach a predetermined turbidity or the rate of turbidity development.

The test is usually performed at 37 ± 1 °C, and the turbidity is expressed in terms of either absorbance or transmission.

2) Chromogenic assay

This method is used to quantify endotoxin by measuring the amount of chromophore groups released from the synthetic chromogenic substrate by the reaction of endotoxin with lysate TS, by absorption or transmittance. This method includes the end-point-chromogenic method and the kinetic-chromogenic method. The end-point-chromogenic test is based on the quantitative relationship between the endotoxin concentration and the quantity of chromophore released after a certain time. The kinetic-chromogenic test measures either the time needed for the reaction mixture to reach a predetermined absorbance or transmittance or the rate of color development.

The test is usually performed at 37 ± 1 °C.

3) Preparatory testing

To ensure the accuracy and validity of the turbidimetric or chromogenic assays, perform both tests for assurance of criteria for the standard curve and for interfering factors.

a) Assurance of criteria for the standard curve

The assurance of criteria for the standard curve should be confirmed before using the lysate reagent for each lot. The test should also be performed when test conditions change, which could affect the test results.

Using the endotoxin standard solution, prepare at least 3 endotoxin concentrations to generate the standard curve within the concentration range of endotoxin specified in the lysate reagent used. Perform the test using at least 3 replicates of each standard endotoxin concentration according to the optimal conditions for the lysate reagent used (with regard to volume ratios, incubation time, temperature, pH, etc.). If the desired range is greater than two logs, additional standards should be included to bracket each log increase in the range of the standard curve. When the absolute value of the correlation coefficient, | r |, must be greater than or equal to 0.980, it is determined that the reliability of the standard curve has been confirmed. If the standard curve does not comply with the test, repeat the test after verifying the test conditions.

b) Test for interfering factors

Prepare solutions A, B, C, and D as shown in Table 4. Perform the test on these solutions following the optimal conditions for the lysate reagent used with regard to volume of test solution and lysate TS, volume ratio of test solution to lysate TS, incubation time, etc. This test should be performed when any condition changes that is likely to influence the result of the test.

Table 4

Solution	Concentration of endotoxin	Solution to which endotoxin is added	Number of test tubes or wells
A[1)]	0	Test solution	≥ 2
B[2)]	Middle concentration of the standard curve[2)]	Test solution	≥ 2
C[3)]	≥ 3 concentrations	Water for BET	≥ 2 for each concentration
D[4)]	0	Water for BET	≥ 2

1) Test solution only (to measure endotoxin concentration in the test solution). The test solution may be diluted to not greater than the MVD.
2) Standard endotoxin is added to the test solution diluted with the same dilution as solution A so that the endotoxin concentration is at or near the middle point of the standard curve.
3) Endotoxin standard solution with various concentrations used for assurance of criteria for the standard curve as described under A) Preparatory testing.
4) Negative control. Water for BET only

The test is valid when the following conditions are met.

Condition 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.

Condition 2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent used, or it is less than the endotoxin detection limit of the lysate reagent used.

Calculate the recovery of the added endotoxin by subtracting the mean endotoxin concentration in solution A from that in solution B, which contains the added endotoxin. The test solution is considered free of interfering factors if under the conditions of the test, the measured concentration of the endotoxin added to the test solution is within 50 - 200% of the known added endotoxin concentration, after subtracting any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified range, the test solution is considered to contain interfering factors. If the test solution does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference with the test solution or diluted test solution that does not exceed the MVD may be eliminated through suitable treatment, such as filtration, neutralization, dialysis, or heat treatment. To determine if the selected treatment effectively removes the interference without losing endotoxins, perform the test described above with the test solution to which the standard endotoxin has been added and which has then been subjected to the selected treatment.

4) Assay

a) Procedure

Prepare solutions, A, B, C, and D according to table 4, and follow the procedure described in 3) Preparatory testing B) Test for interfering factors.

b) Calculation of endotoxin concentration

Calculate the endotoxin concentration of solution A using the standard curve generated with solution C. The test is valid when the following requirements are met.

Condition 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.

Condition 2: The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in solution A, is within the range of 50% to 200%.

Condition 3: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent used, or it is less than the endotoxin detection limit of the lysate reagent used.

c) Interpretation

The sample complies with the test when the endotoxin concentration (EU/mL, EU/mg, EU/mEq, or EU/unit) of the sample, based on the mean endotoxin concentration of solution A, satisfies the endotoxin limit specified in the individual monograph.

Bioautography
바이오오토그래프법

The Bioautography is a method to identify and to assay the active component among substances separated by the paper chromatography or thin-layer chromatography microbiologically. If necessary, the content can usually be specified as% in the specifications of the individual monograph. Unless otherwise specified, perform the test as follows:

Preparation of developing solvent, standard solution, test solution, medium, test microorganism, and test microbial suspension (or test spore solution)

Specified in the monograph.

Preparation of incubation box

Use a stainless steel incubation box with a height of about 25 mm, width of 200 - 300 mm, and length of 350 - 500 mm. When using, sterilize it together with a glass plate.

Stationary phase

1) Paper

Use a piece of paper for paper chromatography with a width of 2 - 14 cm, and length of about 40 cm.

2) Thin-layer plate

Prepare as directed under thin-layer chromatography.

Procedure

Prepare a plate by pouring 200 ~ 300 mL of medium into the culture box. Spread 100 ~ 150 mL of a test microorganism solution (or spore solution) on the plate. Place the culture box at a flat ground and allow to stand for more than 1 hour at not exceeding 5 °C. Perform this procedure aseptically, if possible. Divide the origin line of filter paper or thin-layer plate into 4 equal sections and mark them by vertical lines. Spot 5 μL standard solution, starting from the highest concentration sequentially, on the center of origin line in Sections 1 to 3, and 5 μL test solution on the center of origin line in Section 4 for paper, by using micropipette, and air-dry. If necessary, place the filter paper or thin-layer plate in the chamber saturated with vapor of a developing solvent mixtures for 30 ~ 60 minutes, transfer it to an appropriate chromatographic device, and lower or raise the developing solvent. An operating temperature should be 20 ~ 30 °C. When the front line of developing solvent mixtures approaches 10 ~ 30 mm from the end line of the filter paper or thin-layer plate, take out the filter paper or thin-layer plate, and allow to stand at ordinary temperature for drying. After drying, place the filter paper or thin-layer plate onto agar media in culture box. If necessary, cut each section of filter paper of thin-layer plate and place parallel to each other with 15 mm interval on the agar media in culture box. Keep the filter paper or thin-layer plate close contact with agar media for 5 to 15 minutes, and remove the filter paper of thin-layer plate from the media. Avoid a contamination during this manipulation. Unless otherwise specified, incubate the culture box at 32 ~ 37℃ for 17 ~ 20 hours.

Developing distance, R_f value, measurement, calculation, and evaluation

Follow each monograph.

Boiling Point and Distilling Range
비점측정법 및 증류시험법

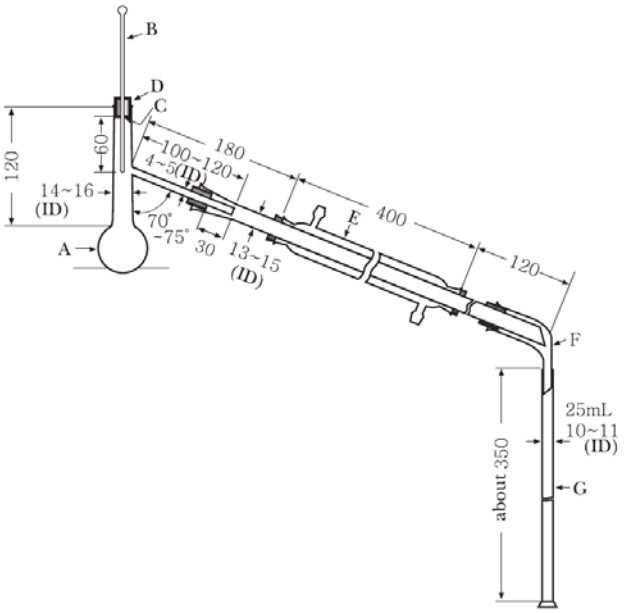
The Boiling Point and Distilling Range is performed by the following Method 1 or Method 2 unless otherwise specified. The boiling point is set as the temperature between when the first 5 drops of distillate are leaked from the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is performed to determine the volume of distillate collected at the temperature range specified in the monograph.

Method 1

If the specified temperature range is less than 5 °C

1) Apparatus

Use the apparatus shown in the figure.



* The figures are in mm.

Figure

A: Distilling flask B: Thermometer with immersion line
C: Immersion line D: Cork stopper
E: Condenser F: Adapter
G: Stoppered measuring cylinder (25 mL, graduation: 0.1 mL)

2) Procedure

Take 25 mL of the sample, whose temperature was previously recorded, using a measuring cylinder G with 0.1 mL graduation and transfer it into the distilling flask A with the capacity of 50 mL to 60 mL. Use this cylinder for the distillate without rinsing. Add boiling stones to A and ensure that the immersion line C is at the lower end of the cork stopper D and the upper end of its mercury bulb is in the center of the delivery tube in the thermometer with immersion line B. Connect the condenser E to A and insert the adapter F to E. Insert the end of F into the opening of measuring cylinder G to let some air pass through. Set up a windbreak enough to enclose A and heat it with a suitable heat source. However, when heating with direct fire, put A on top of the hole in the heat-resistant insulating material plate [150 mm × 150 mm heat-resistant insulating material plate (or 150 mm × 150 mm metal mesh attached with 6 mm thick heat-resistant insulation material) with about 6 mm thick and 30 mm diameter circular hole in the center]. Unless otherwise specified, the leak rate when distilling is 4 mL - 5 mL per minute for liquids whose boiling temperature to be determined is lower than 200 °C, and 3 mL to 4 mL per minute for liquids whose boiling temperature is NLT 200 °C, to read the boiling point. In the distilling range test, measure the volume of distillate after ensuring that the temperature of the solution is the same as the initial temperature of the sample. For liquids that begin to be distilled at 80 °C or below, cool the sample in advance to 10 - 15 °C before measuring the volume, and during distillation, cool the measuring cylinder at 25 mm or below from the top with ice. Temperature correction on the atmospheric pressure is performed by 0.1 ℃ per 0.36 kPa, which is added when the atmospheric pressure is less than 101.3 kPa, and subtracted when it is greater than 101.3 kPa.

Method 2

If the specified temperature range is NLT 5 °C

1) Apparatus

Use the same apparatus as described in Method 1. However, use a 200 mL distilling flask A, where the delivery tube that the internal diameter of the neck is 18 mm - 24 mm and its internal diameter is 5 mm - 6 mm is attached. And for direct-fire heating, use a heat-resistant insulating material plate with a round hole of 50 mm in diameter in the center.

2) Procedure

Take 100 mL of the sample whose temperature was measured in advance using a measuring cylinder graduated in 1 mL and perform the same procedures as in Method 1.

Characterization of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction

분말 X 선 회절측정법

The Characterization of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction is performed to measure the diffraction intensity of coherent scattering of X-rays at each diffraction angle when irradiating a powder sample with X-rays and forcing electrons in the material to vibrate. Each crystalline phase of the compound shows a characteristic X-ray diffraction pattern. X-ray diffraction patterns can be obtained from randomly oriented crystalline powder composed of crystallites (crystalline regions within the grain) or crystal fragments of a defined size. In a powder diffraction pattern, the following three types of information can basically be obtained from the X-ray diffraction patterns: the angle of the diffraction lines (depending on the structure and size of the unit lattice), the intensities of the diffraction lines (mainly depending on the type and arrangement of the atoms and the orientation of the particles in the sample), and the shapes of the diffraction lines (depending on the instrument resolution, crystallite size, deformity, and sample thickness). Measurement of the angle and intensity of the diffraction line can be used for qualitative and quantitative phase analysis, such as identifying the crystal structure of crystalline materials. It is also possible to evaluate the ratio of amorphous to crystalline fractions.[1)] Unlike other analytical methods, X-ray powder diffraction (XRPD) has the advantage of being a non-destructive measurement method (sample preparation is usually limited to grinding to obtain a randomly oriented sample). X-Ray powder diffraction can also be performed to measure samples under conditions other than normal conditions, such as low or high temperatures and humidity.

1. Principle

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on atom arrangement, interference occurs at the elastically scattered X-rays. The interference gets stronger when the path difference between 2 diffracted X-ray waves is an integer multiple of wavelength. This selective condition is described by the Bragg equation, which is also called Bragg's law (see Fig. 1).

$$2d_{hkl}\sin\theta_{hkl} = n\lambda$$

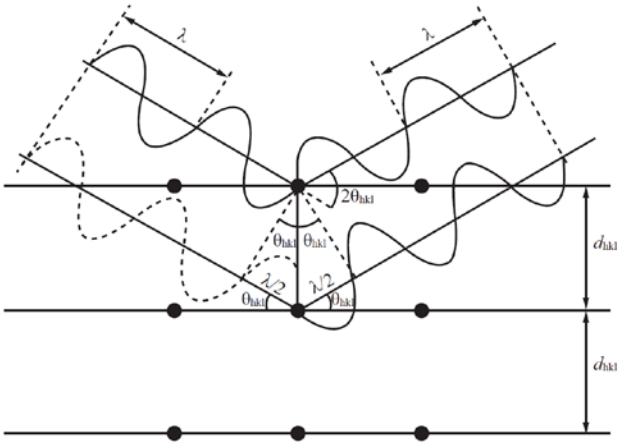


Figure 1. Diffraction of X-rays by a crystal according to Bragg's law.

The wavelength λ of the X-rays is about the size of the distance between successive crystal lattice planes, or *d*-spacing (d_{hkl}). θ_{hkl} is the angle between the incident X-ray and the family of lattice planes, and $sin\theta_{hkl}$ is inversely proportional to the distance between successive crystal planes or d-spacings d_{hkl}.

The direction and spacing of the planes with respect to the unit lattice axis are defined by the Miller indices (*hkl*). These indices are the reciprocals, reduced to the next lower integer, of the intercepts that a plane makes with the unit lattice axis. The unit lattice dimensions are given by the axis lengths, a, b, and c, and the angles between the axis and each of them, α, β, and ν. The spacing between the planes for a specified set of parallel *hkl* planes is denoted as d_{hkl}. The planes of the same family of each lattice plane have a d-spacing of 1/n (n is an integer) and show high diffraction orders along the *nh*, *nk*, and *nl* planes. Each plane unit of a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with a certain wavelength λ. For polycrystalline powder samples, there are always crystallites in an orientation that enables diffraction according to Bragg's law at any angle θ_{hkl}[2)]. For a certain wavelength of X-rays, the positions of the diffraction peaks (also called diffraction lines, reflections, or Bragg reflections) are characteristic of the crystal lattice (*d*-spacings). Their theoretical intensities depend on the content (type and position of atoms) of the crystallographic unit lattice, and the line profiles on the perfection of crystal lattice or the size of crystals. Under these conditions, the diffraction peak intensity depends on the atom arrangement, the type of atoms, thermal motion and structural imperfections, and the characteristics of the measuring instrument. The intensity of diffraction depends on many factors including structure factor, temperature factor, crystallinity, polarization factor, multiplicity, Lorentz factor and micro-absorption factor. Major characteristics of the diffraction pattern are the 2θ-position, peak height, peak area, and peak shape (e.g., characterized by peak width or asymmetry, analytical function, and empirical representation). Figure 2 shows an example of X-ray powder diffraction pattern obtained from 5 different stationary phases of a certain material.

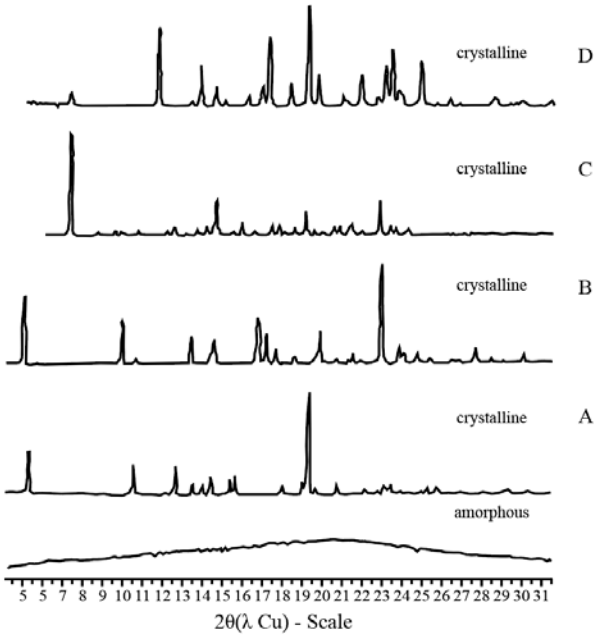


Figure 2. X-ray powder diffraction patterns obtained from 5 different solid phases of a certain material (The intensities of crystalline forms A-D are adjusted to the same scale.)

Measurement of X-ray powder diffraction also produces some uniform background on which the peaks are superimposed. In addition to the method of sample preparation, background causes also include other factors such as diffuse scattering by sample holder, air, sample and equipment; noise in the detector, and other instrumental parameters including general radiation from the X-ray tube. The peak to background ratio can be increased by minimizing background and by extending exposure duration.

2. Apparatus

1) Composition

For measurement of X-ray diffraction, a powder diffractometer or powder camera is used. A powder diffractometer generally consists of 5 main parts: X-ray source; incident light optics for monochromating, filtering, parallelizing or focusing of light; goniometer; diffracted light optics for monochromating, filtering, parallelizing or focusing of light; and detector. Separately, an X-ray diffractometer requires a system for data collection and processing, and it is usually mounted on the diffractometer. Depending on the purpose of analysis (phase identification, quantitative analysis, determination of lattice parameters, etc.), the composition or performance level of the required apparatus will vary. The simplest apparatus for measuring powder diffraction patterns is a powder camera. By replacing the photographic film with a photon detector during detection, the diffractometer is designed not to actually focus, but to parafocus, like the Bragg-Brentano geometry, through the geometrical arrangement of the optics. The Bragg-Brentano parafocusing system is currently the most widely used and is therefore briefly described here.

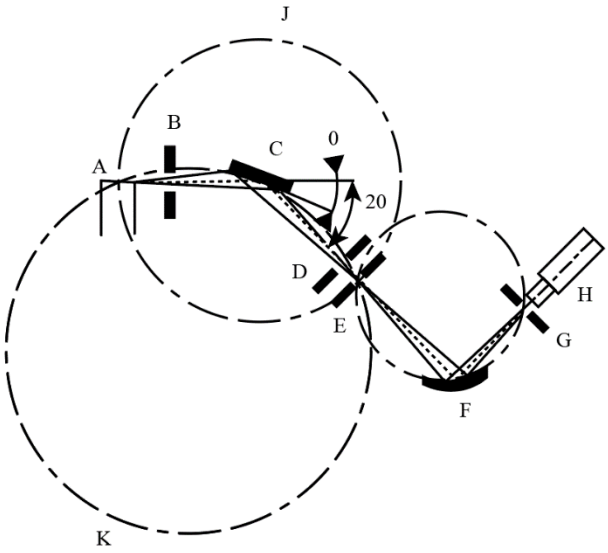


Figure 3. Geometrical layout of Bragg-Brentano parafocusing system

A: X-ray tube	B: Divergence slit
C: Sample	D: Anti-diffusion slit
E: Receiving slit	F: Monochromator
G: Detector receiving slit	H: Detector
J: Diffractometer circle	K: Focusing circle

A given apparatus may provide a horizontal or vertical θ/2θ geometry or vertical θ/θ geometry. In either geometry, the incident X-ray beam forms an angle θ with the surface plane of the sample, while the diffracted X-ray beam forms an angle θ with the surface plane of the sample, but has an angle 2θ with the direction of the incident X-ray beam. The basic geometric arrangement is presented in Fig. 3. The divergent beam (primary beam) of radiation from the X-ray tube passes through a Soller slit and a divergence slit, and is incident on the flat surface of the sample. All X rays diffracted by appropriately oriented crystallites in the sample at an angle of 2θ converge to a line at the receiving slit. A second set of Soller slit and scatter slit may be placed either behind or before the receiving slit. Typically, the second light receiving slit is used only when there is a 0D detector. The axes of the X-ray tube line focus and of the receiving slit are at equal distances from the axis of the goniometer. X-rays are measured by a detector, usually a scintillation counter or a sealed gas proportional counter. However, detectors that are currently more common are position-sensitive solid-state detectors or hybrid photon counting detectors. The receiving slit device and the detector are coupled together and move tangentially to the focusing circle. For $\theta/2\theta$ scans, the goniometer rotates the specimen about the same axis as that of the detector, but the samples rotate at half the rotational speed of the detector. The surface of the sample thus remains tangential to the focusing circle. The Soller slit limits the axial divergence of the beam, and thus partially controls the shape of the diffraction line profile. A diffractometer can also be used in transmission mode. The advantage of this method is to reduce the effects of preferential alignment. A capillary tube about 0.5 to 2 mm in thickness can also be used for small sample amounts.

2) X-ray radiation

In the laboratory, X-rays are obtained by accelerating electrons, which are emitted by thermionic effect, in a strong electric field using a high-voltage generator to a metal anode. Since most

of the kinetic energy of electrons is converted into heat, the anode needs to be sufficiently cooled to maintain the function of the X-ray tube. A brightness can be increased 20 to 30 times by using rotating cathodes and optimized X-ray optics. Alternatively, X-ray photons can be generated in a large-scale facility such as synchrotron, an electron accelerator. The spectrum emitted by an X-ray tube operating at high voltage consists of a continuous spectrum of polychromatic radiation (background) and additional specific X-rays determined by the type of anode. Generally, only specific X-rays are used for X-ray diffraction measurements. Major radiation sources used for X-ray diffraction are vacuum tubes with copper, molybdenum, iron, cobalt, silver or chromium anodes. X-rays of copper and molybdenum are commonly used for X-ray diffraction measurement in organic substances.

The choice of radiation to be used depends on the absorption properties of the specimen and the possible fluorescence of the atoms present in the specimen. The wavelength used in X-ray powder diffraction is usually K_α ray, generated from a polar ray. Therefore, it is necessary to make the X-ray beam monochromatic by removing all components of the emission spectrum other than K_α. This can be partly achieved using K_β filters, i.e., metal filters selected as having an absorption edge between the K_α and K_β wavelengths emitted by the X-ray tube.

Such a filter is usually inserted between the X-ray tube and the sample. A more commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as monochromator). This crystal is placed before or behind the specimen and diffracts the different characteristic peaks of the X-ray beam (i.e. K_α and K_β) at different angels, so that only one of them may be selected to enter into the detector. It is even possible to separate the $K_{\alpha1}$ and $K_{\alpha2}$ rays using a special monochromator. However, when monochromatic light is obtained using a filter or a monochromator, its intensity is reduced. Another way to separate K_α and K_β wavelengths is to use curved X-ray mirrors, and this can achieve monochrome, focus adjustment or parallelizing of X-ray beam simultaneously.

3) Protection against radiation

Exposure of any part of the human body to X-rays can be harmful to health. Therefore, proper precautions must be taken when using an X-ray, to protect the operator and anyone in the vicinity. Each country establishes acceptable standards for X-ray exposure levels or recommendations for protection against radiation in accordance with their national laws. In the absence of official regulations or recommendations, the latest recommendations of the International Commission on Radiological Protection should be applied (required training on protection against radiation and acceptable standards for X-ray exposure levels are governed by the Nuclear Safety Act).

3. Sample preparation and filling

Preparation of the specimens/samples and filling the samples into an appropriate holder are critical steps in many analytical methods, especially in X-ray powder diffraction, as they can significantly affect the quality of the data being collected.[3] Major causes of errors during sample preparation and filling when using Bragg-Brentano parafocusing system are briefly stated below.

1) Specimen preparation

In general, the morphology of most crystalline particles tends to give a sample with some preferred orientation in the specimen holder. This is particularly evident for needle-like or plate-like crystals when size reduction yields finer needles or platelets. The preferred orientation in a specimen affects the intensities of the various reflections, which results in some more intense or weaker reflections than those from a completely random sample. There are several methods that can be used for the randomness of the crystallite orientation (and thus minimize the preferred orientation), but the best and easiest way is to reduce the particle size. The optimal number of crystallites depends on the geometry of the diffractometer, the required resolution, and the attenuation of the X-ray beam according to the sample. In some cases, the image can be sufficiently confirmed at a particle size of about 50 μm. However, excessive pulverization (particle sizes of less than about 0.5 μm) can lead to line broadening and significant changes in the sample itself, such as:

(i) contamination of the specimen by particles rubbed off by the pulverizing devices (mortar, pestle, balls, etc.)
(ii) reduced degree of crystallinity
(iii) solid phase transition to another polymorph
(iv) chemical decomposition
(v) development of internal stress
(vi) solid-state reactions

Therefore, it is desirable to compare the diffraction patterns of non-ground specimens and ground specimens (with a small particle size). If the X-ray powder diffraction pattern obtained is of sufficient quality for the intended use, grinding may not be necessary. Precautions must be taken since the original composition may change if a sample contains multiple phases and if the particles are isolated by sieving to a specific size.

2) Specimen filling

a) Specimen displacement effect: A specimen surface offset by D with respect to the rotation axis of the diffractometer causes systematic errors that are very difficult to completely avoid. In reflection mode, a movement of the absolute value $D\cdot\cos\theta$[4] occurs at the 2θ position (typically about 0.01° per 2θ at low angles ($\cos\theta\approx1$) for displacement D = 15 μm), and the profile broadens asymmetrically toward low 2θ values. Using an appropriate internal reference material allows you to detect the effects of sample transparency and correct accordingly. These effects are the biggest cause of errors in data collected with properly placed diffractometers.

b) Specimen thickness and transparency effects: For powder X-ray diffractometry in reflection mode, it is often preferred to work with “infinite thickness” samples. Non-diffracting substrates (background-free holders) can minimize transparency effects.[5] An example is a single crystal silicon plate cut parallel to the 510 lattice plane. One of the advantages of transmission mode is that sample height and sample transparency are not very important. Using an appropriate internal reference material allows you to detect the effects of sample displacement and correct accordingly.

4. Management of apparatus performance

Goniometers and the corresponding optics for the incident and diffracted X-ray beam have several mechanical parts that must be adjusted. The degree of alignment or misalignment directly affects the quality of the XRPD measurement results. Therefore, to adequately minimize systematic errors, various components of the diffractometer, such as optical and mechanical systems, must be carefully adjusted to obtain the optimum X-ray intensities at the detector. When adjusting a diffractometer, it is not easy to determine the maximum intensity and maximum resolution at the same time. Therefore, the optimal condition must be determined by adjusting the apparatus. There are many different alignment methods, and each instrument requires specific alignment procedures. The performance of the overall diffractometer must be regularly tested and inspected using reference materials (silicon powder or α-aluminum (corundum)). In this

case, certified reference materials are desirable, but other materials may be used depending on the type of analysis.

5. Qualitative phase analysis (identification of phase)

Identification of each phase in an unknown sample by XRPD is usually based on visual or computer-assisted comparison of a portion of the X-ray diffraction pattern of the powder with the experimental or calculated pattern of a reference material. Ideally, these reference patterns are collected on well-characterized single-phase specimens. In most cases, this approach allows for the identification of crystalline substances by the 2θ diffraction angles or *d*-spacings and relative intensities. When comparing the diffraction pattern of an unknown sample and reference data using a computer, either the entire diffraction pattern in the 2θ range, or the main part of the diffraction pattern can be used. For example, the table of surface spacing d and standardized intensity Inorm obtained from each diffraction pattern, the so-called (*d*, Inorm) table, serves as a fingerprint of a crystalline material, and can be compared with the (*d*, Inorm) table for single phase samples in the database. For the measurement of the most organic crystals using CuK_α radiation, it is appropriate to record the diffraction pattern in a 2θ range from as close to 0°, as near to possible, to at least 30°. The 2θ diffraction angles between the same crystal structure specimen and reference material are within 0.2°. However, the relative intensities between the sample and reference material can vary significantly due to preferred orientation effects. By their nature, hydrates or solvates that readily transition are known to change the dimension of the lattice, and in this case, there is a shift in the peak position in the diffraction pattern. In these materials, a deviation of 2θ positions of greater than 0.2° is expected. Therefore, the peak position deviation of 0.2° is not applicable. For other types of samples (e.g., inorganic salts), it may be necessary to extend the 2θ range scanned to NLT 30°. In general, it is sufficient to measure reflections with intensity NLT 10 times listed in the powder X-ray diffraction database of single-phase samples.

In the following cases, it is sometimes difficult or even impossible to identify phases:

(i) non-crystallized or amorphous substances

(ii) the components to be identified are present in low mass fractions of the analyte amounts (generally less than 10%)

(iii) pronounced effects of the preferred orientation

(iv) the phase is not listed in the database used

(v) formation of solid solutions

(vi) presence of disordered structures that alter the unit lattice

(vii) sample with too many phases

(viii) presence of lattice deformations

(ix) structural similarity of different phases

6. Quantitative phase analysis

If the sample to be analyzed is a mixture of multiple known phases, NMT 1 of which is amorphous, the percentage (by volume or by mass) of each crystalline phase or the amorphous phase can be determined in many cases. Quantitative phase analysis is based on the integrated intensities and the peak heights and overall pattern of several individual diffraction lines[6]. These integrated intensities, peak heights, or data points of the entire pattern are compared with the corresponding values of reference materials. These reference materials should be a single phase or a mixture of known phases. Specimen preparation (among the samples, the homogeneity of all phases and a suitable particle size distribution in each phase are required for the accuracy and precision of the results) and matrix effects are problems in the quantitative phase analysis. In general, up to 10% of the crystalline phase in the solid matrix can be measured, and in better cases, less than 10% can be quantified.

1) Polymorphic samples

In a sample consisting of 2 polymorphic phases *a* and *b*, the following expression may be used to quantify the fraction F_a of phase *a*:

$$F_a = \frac{1}{1 + K(\frac{I_b}{I_a})}$$

The fraction can be determined by measuring the intensity ratio between the 2 phases and by obtaining the constant *K*. *K* is the ratio of the absolute intensities of the 2 pure polymorphic phases I_{oa}/I_{ob}, which can be determined by measuring reference materials.

2) How to use reference materials

The most commonly used methods for quantitative analysis are:

- external standard method,
- internal standard method,
- spiking method (or standard addition method).

The external standard method is the most general method that compares the X-ray diffraction pattern or the peak intensity of the mixture to be measured to those measured with a mixture of reference materials. It is also possible to determine the amount by comparing the theoretical intensities of a structural model, if the structure is fully known. The internal standard method is the method most commonly used because its diffraction pattern does not overlap with the components of the sample to be measured, and an internal reference material with the same crystallite size or X-ray absorption coefficient reduces errors through the matrix effect. A known quantity of this reference material is added to the sample and to each of the reference mixtures. Under these conditions, there is a linear relationship between line intensity and concentration. In the internal standard method, it is necessary to measure the diffraction intensities accurately. In the standard addition method, a specific amount of phase a is added to a mixture containing phase a at an unknown concentration. When creating a calibration curve of the relationship between concentration and strength by preparing several samples with different amounts of addition, the negative intercept value on the x-axis becomes the concentration of phase a in the mixture.

7. Evaluating percentages of the amorphous and crystalline fractions

There are several methods that can determine the percentages of crystalline and amorphous phases in a mixture of crystalline and amorphous phases. The choice of the measurement method used depends on the nature of the sample:

(i) When a sample consists of multiple crystalline fractions and an amorphous fraction, the amount of the individual crystalline phases can be estimated using suitable reference materials as described above; the amount of the amorphous fraction is then determined indirectly by subtraction.

(ii) When a sample consists of 1 crystalline and 1 amorphous fraction of the same elemental composition, the amount of the crystalline phase (degree of crystallinity) for 1-phase or even a mixture of 2 phases, can be estimated by measuring 3 areas of the diffractogram.

A = Total area of the peaks from the diffraction of the crystalline part of the sample

B = Area under the curve in the diffractogram generated by the sample (excluding the area of *A*)

C = Background area (depends on scattering by air, fluorescence, equipment, etc.)

Once these areas have been measured, the degree of crystallinity can be roughly estimated using the following formula.

$$\text{Degree of Crystallinity (\%)} = 100A / (A + B - C)$$

It should be noted that this method does not provide an absolute degree of crystallinity and is, therefore, generally used for comparative purposes only. There are also more sophisticated methods, such as the Ruland method.

8. Interpretation of a single crystal structure

In general, the determination of crystal structures is based on X-ray diffraction data obtained from single crystals. However, in the organic crystal, the lattice parameters are comparatively large, the symmetry is low, and the scattering properties are usually not very remarkable, so interpretation of the structure is not easy. If the crystal structure of a substance is already known, the corresponding XRPD pattern can be calculated, thereby providing a preferred-orientation-free reference XRPD pattern that can be used for phase identification.

1) Applications of X-ray powder diffraction include crystalline pharmaceutical substances such as the determination of crystal structures, refinement of crystal structures, determination of crystallographic purity of crystalline phases, and characterization of crystallographic texture. There are many other examples, but they are not described in detail in this chapter.
2) An "ideal" powder for X-ray diffraction measurement consists of a large number of small, randomly oriented spherical particles (coherently diffracting crystalline domains). If the number of crystallites is sufficient, reproducible diffraction patterns can be obtained in any diffracting orientation.
3) The properties of the sample in a non-equilibrium state may change during measurement due to conditions such as the influence of temperature and humidity.
4) Caution must be exercised as goniometer zero alignment shifts result in constant shifts in all observed 2θ line positions. In this case the entire diffraction pattern is offset-transformed from 2θ to Z°.
5) Thin samples with low attenuation allow for accurate measurement of line positions using focused diffractometers in either transmission or reflection geometries. It is desirable to use a parallel beam optical spectrometer to accurately measure the line position of a sample with a low attenuation rate. This can reduce the influence of sample thickness.
6) If the crystal structures of all components are known, quantitative analysis with a high degree of accuracy is possible using the Rietveld method. If the crystal structures are not known, the Pawley method or the least squares method can be used.

Chloride
염화물시험법

The Chloride is to test the limit for chloride contained in drugs.

In the monograph, the limit for chloride (as Cl) is given in terms of percentage (%) in parentheses.

Procedure

Unless otherwise specified, put the amount of sample indicated in the monograph in a Nessler tube and dissolve it in the proper amount of water to prepare a 40 mL solution. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Separately, put the amount of 0.01 mol/L hydrochloric acid indicated in the monograph in another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the blank. If the test solution is not clear, filter both solutions under the same conditions.

Add 1 mL of silver nitrate TS to the test and control solutions, and mix well. Keep the solution away from direct sunlight and allow it to stand for 5 minutes. Then compare turbidity of these solutions from the top or side of the Nessler tube against a black background.

The turbidity developed in the test solution is not thicker than that of the control solution.

Chromatography
크로마토그래피 분리분석 총론

1. Introduction

Chromatographic separation techniques are multi-stage procedures in which the components of a sample are distributed between 2 phases-one stationary and other mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. It can be packed in a column, spread as a layer, or distributed as a film among other methods. The mobile phase may be gaseous or liquid or supercritical fluid. Separation can be achieved through adsorption, mass distribution (partition), ion exchange, etc., or differences in the physicochemical properties of the molecules such as size, mass, volume, etc.

This chapter provides definitions and calculations of common parameters and generally applicable requirements for system suitability. Principles of separation, apparatus and methods are detailed in the corresponding general tests.

–57. Paper Chromatography
–78. Thin Layer Chromatography
–33. Gas Chromatography
–43. Liquid Chromatography
–72. Size-exclusion Chromatography

2. Definitions

The system suitability and acceptance criteria in monographs are established using the parameters as defined below. With certain equipment, specific parameters such as the signal-to-noise ratio and resolution can be calculated using software provided by the manufacturer. It is the user's responsibility confirm that the calculation methods used in the software are equivalent to the requirements of the Korean Pharmacopoeia. If not, necessary correction must be made.

1) Chromatogram

A graphical or alternative representation of detector response, effluent concentration or another quantity used as a measure of effluent concentration, versus time or volume. Idealised chromatograms are represented as a sequence of Gaussian peaks on a baseline (Figure 1).

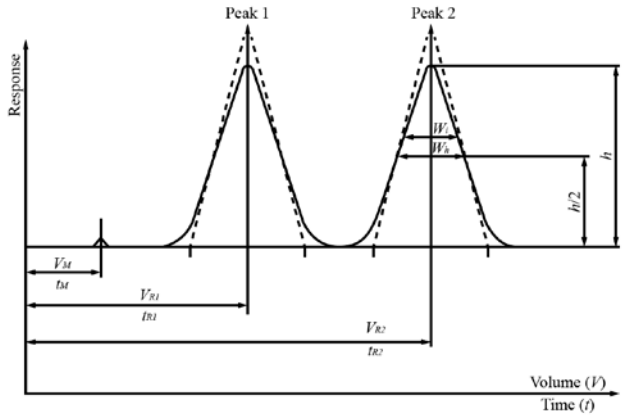


Figure 1

V_M = Hold-up volume
t_M = Hold-up time
V_{R1} = Retention volume of peak 1
T_{R1} = Retention time of peak 1
V_{R2} = Retention volume of peak 2
t_{R2} = Retention time of peak 2
W_h = Peak width at half-height
W_i = Peak width at the inflexion point
h = Height of the peak
$h/2$ = Half-height of the peak

2) Distribution constant (K_0)

In the Size-exclusion Chromatography, the elution characteristics of a component in a specific column can be expressed by the distribution constant (also known as the distribution coefficient) calculated using the following equation:

$$K_0 = (t_R - t_0)/(t_t - t_0)$$

t_R = Retention time
t_0 = Retention time of an unretained compound
t_t = Total mobile phase time

3) Dwell volume *(D)* (also referred to as V_D)

The dwell volume also known as gradient delay volume, represents the volume between the point where the eluents meet and the inlet of the column. It can be determined using the following procedure.

Column: replace the chromatographic column with an appropriate capillary tube (e.g. 1 m x 0.12 mm).

Mobile phase:

mobile phase A: water

mobile phase B: 0. 1 vol% solution of acetone in water

Time (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 ~ 20	100 → 0	0 → 100
20 ~ 30	0	100

Flow rate: Set to obtain sufficient back-pressure (e.g. 2 mL/min).

Detection: spectrophotometer at 265 nm.

Determine the time *($t_{0.5}$)* in minutes when the absorbance has increased by 50% (Figure 2).

$$D = t_D \times F$$

$t_D = t_{0.5} - 0.5t_G$, in minutes
t_G = pre-defined gradient time (= 20 mins)
F = flow rate, in millilitres per minute

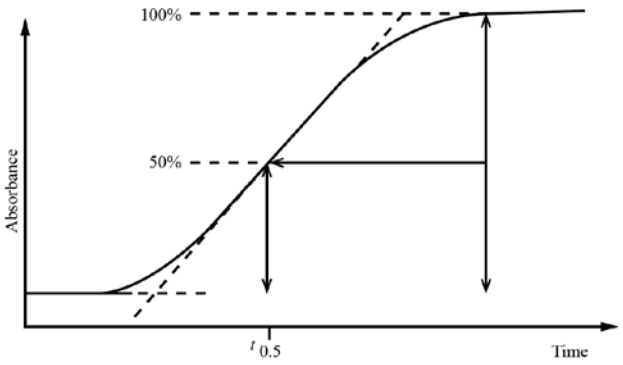


Figure 2

Note: where applicable, this measurement is performed with the autosampler in the inject position so as to include the injection loop volume in the dwell volume.

4) Hold-up time ™

Time required for elution of an unretained component (Figure 1, baseline scale being in minutes or seconds).

In size-exclusion chromatography, the term retention time of an unretained compound *(t_0)* is used.

5) Hold-up volume (V_M)

Volume of the mobile phase required for the elution of an unretained component. VM may be calculated from the hold-up time ™ and the flow rate *(F)*, expressed in millilitres per minute using the following equation:

$$V_M = t_M \times F$$

In size-exclusion chromatography, the term for the retention volume of an unretained compound (V_0) is used.

7) Peak

A portion of a chromatogram recording the detector response when a single component (or 2 or more unresolved components) is eluted from the column.

The peak response may be represented by the peak area or the peak height *(h)*.

8) Peak-to-valley ratio *(p/v)*

The peak-to-valley ratio may be employed as a system suitability criterion when baseline separation between two peaks is not achieved (Figure 3).

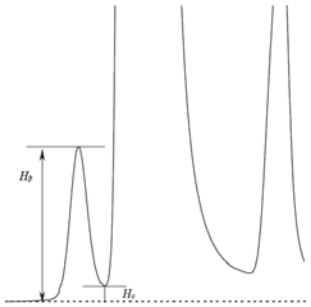


Figure 3

$$p/v = H_p / H_v$$

H_p = height above the extrapolated baseline of the minor peak

H_v = height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks.

8) Plate height (H) (synonym: Height equivalent to one theoretical plate (HETP))

Ratio of the column length *(L)*, in micrometers, to the plate number *(N)*:

$$H = L/N$$

9) Plate number (N) (synonym: Number of theoretical plates)

A number indicative of column performance (column efficiency). It can only be calculated from data obtained under either isothermal, isocratic or isodense conditions, depending on the technique. The plate number is calculated using the following equation, with the values of t_R and w_h expressed in the same units:

$$N = 5.54(t_R/ w_h)^2$$

t_R = retention time of the peak corresponding to the component

w_h = peak width at half-height (h/2)

The plate number varies with the component as well as with the column, the column temperature, the mobile phase and the retention time.

10) Reduced plate height *(h)*

Ratio of the plate height *(H)*, in micrometers, to the particle diameter (d_p) in micrometers:

$$h = H / d_p$$

11) Relative retardation *(R rel)*

The relative retardation, used in thin-layer chromatography, is calculated as the ratio of the distances travelled by the spot of the compound of interest and a reference compound (Figure 4).

$$R\ rel = b / c$$

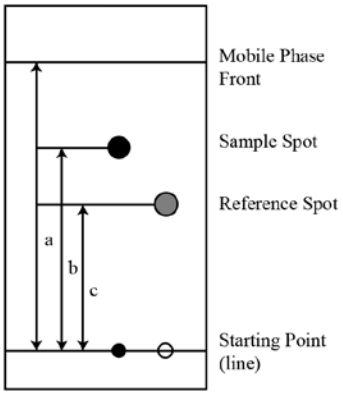


Figure 4

a = migration distance of the mobile phase;
b = migration distance of the compound of interest;
c = migration distance of the reference compound.

12) Relative retention *(r)*

Relative retention is calculated using the following equation:

$$r = (t_{Ri} - t_M) / (t_{Rst} - t_M)$$

t_{Ri} = Retention time of the peak of interest

t_{Rst} = Retention time of the reference peak (usually the peak corresponding to the substance to be examined)

t_M = Hold-up time

13) Relative retention, unadjusted *(r_G)*

Unadjusted relative retention is calculated using the following equation:

$$r_G = t_{Ri} / t_{Rst}$$

Unless otherwise specified, values for relative retention stated in monographs correspond to unadjusted relative retention.

In planar chromatography, retardation factors R_{Fst} and R_{Fi} are used instead of t_{Rst} and t_{Ri}. R_{Fst} (also known as R_{st}) indicates the ratio of the distance travelled by a substance to the distance travelled by a reference substance.

14) Resolution *(R_s)*

The resolution between peaks of two components (Figure 1) is calculated using the following equation:

$$R_S = 1.18(t_{R2} - t_{R1}) / (w_{h1} + w_{h2})$$

$t_{R2} > t_{R1}$

t_{R1}, t_{R2} = Retention times of the peaks

W_{h1}, W_{h2} = Peak widths at half-height

Complete separation means a resolution of NLT 1.5, and is also referred to as baseline separation.

In quantitative thin-layer chromatography, using densitometry, the migration distances are used instead of retention times to calculate the resolution between peaks of two components with the following equation:

$$R_S = 1.18a(R_{F2} - R_{F1})/ (w_{h1} + w_{h2})$$

$R_{F2} > R_{F1}$

R_{F1}, R_{F2} = Retardation factors of the peaks

W_{h1}, W_{h2} = Peak widths at half-height

a = Migration distance of the solvent front

15) Retardation factor *(R_F)*

The retardation factor, (also known as retention factor (R_f)), used in thin-layer chromatography, is the ratio of the distance from the point of application to the center of the spot and the distance simultaneously travelled by the solvent front from the point of application (Figure 4).

$$R_F = b / a$$

b = migration distance of the compound of interest
a = migration distance of the solvent front

16) Retention factor *(k)*

The retention factor (also known as mass distribution ratio *(D_m)* or capacity factor *(k))* is defined as:

$$k = \frac{amount\ of\ component\ in\ stationary\ phase}{amount\ of\ component\ in\ mobile\ phase} = K_c \times (V_S/V_M)$$

K_C = Distribution constant (also known as equilibrium distribution coefficient)
V_S = Volume of the stationary phase
V_M = Volume of the mobile phase

The retention factor of a component may be determined from the chromatogram using the following equation:

$$k = (t_R - t_M) / t_M$$

t_R = retention time
t_M = hold-up time

17) Retention time *(t_R)*

Retention time is the time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone (Figure 1, baseline scale being in minutes or seconds).

18) Retention volume *(V_R)*

Retention volume is the volume of the mobile phase required for the elution of a compound. It may be calculated from the retention time *(t_R)* and the flow rate *(F)* (mL/minute) using the following equation:

$$V_R = t_R \times F$$

19) Retention time of an unretained compound *(t_0)*

In the Size-exclusion Chromatography, the retention time refers to the duration that a component with molecules larger than the maximum gel pore size stays in the system (Figure 5).

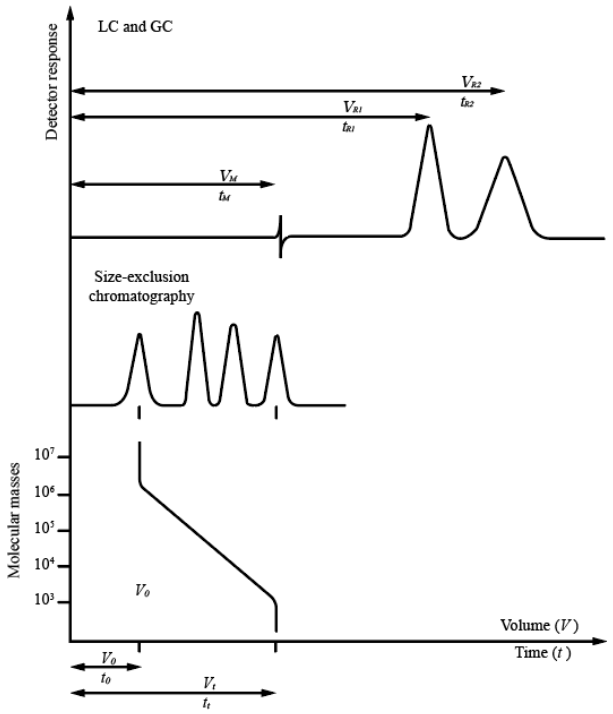


Figure 5

20) Retention volume of an unretained compound *(V_0)*

In size-exclusion chromatography, retention volume of a component whose molecules are larger than the largest gel pores. It can be calculated from the retention time of an unretained compound *(to)* and the flow rate *(F)* (mL/minute) using the following equation:

$$V_0 = t_0 \times F$$

21) Separation factor *(α)*

Relative retention calculated for two adjacent peaks (by convention, the separation factor value is always > 1):

$$\alpha = k_2 / k_1$$

k_1 = Retention factor of the first peak
k_2 = Retention factor of the second peak

22) Signal-to-noise ratio *(S/N)*

The short-term noise can affect the precision and accuracy of quantitation. The signal-to-noise ratio is calculated using the following equation:

$$S/N = 2H/h$$

H = Height of the peak (Figure 6) corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution. It is measured from the peak's maximum to the extrapolated baseline over a distance equal to 20 times the width at half-height.

h = Range of the noise in a chromatogram obtained after injection of a blank (Figure 7), observed over a distance equal to 20 times the width at half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

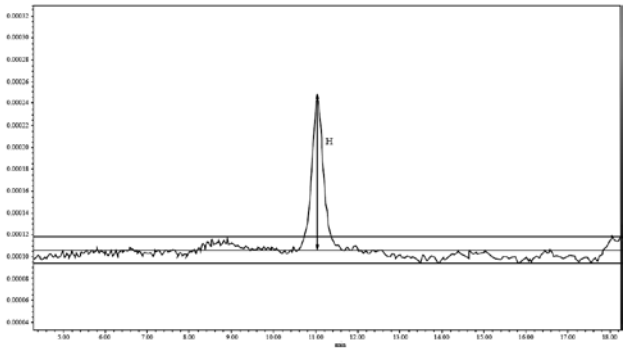


Figure 6. Chromatogram of the reference solution

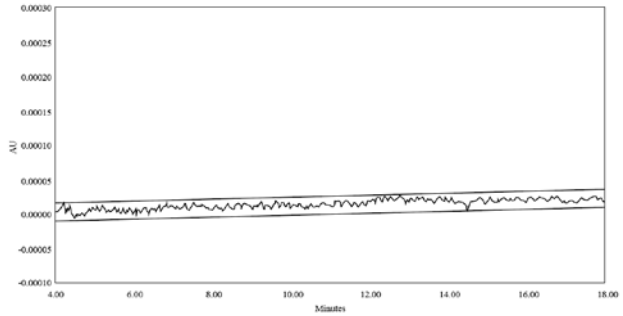


Figure 7. Chromatogram of a blank

If obtaining a baseline at 20 times the width at half-height is not possible due to peaks caused by solvents, reagents, the mobile phase, sample matrix, or the gas chromatographic temperature program, a baseline of at least 5 times the width at half-height is acceptable.

23) Symmetry factor *(A_S)*

The symmetry factor of a peak, also known as the asymmetry factor or tailing factor (Figure 8), is calculated using the following equation:

$$A_S = W_{0.05}/2d$$

$W_{0.05}$ = Width of the peak at one-twentieth of the peak height

d = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height

A_S = 1 signifies symmetry. When A_S > 1.0, the peak is tailing. When A_S < 1.0, the peak is fronting.

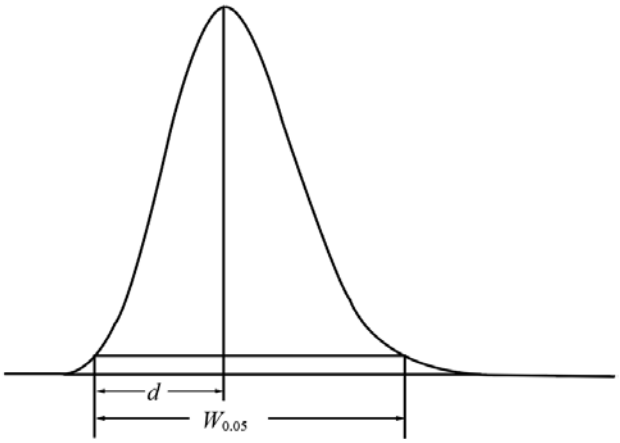


Figure 8

24) System repeatability

The repeatability of the response is expressed as the estimated percentage relative standard deviation (%RSD) of a consecutive series of measurements, involving not fewer than 3 injections or applications of a reference solution. It is calculated using the following equation:

$$\%\text{RSD} = \frac{100}{\bar{y}}\sqrt{\frac{\Sigma(y_1-\bar{y})^2}{n-1}}$$

Y_i = Individual values expressed as peak area, peak height, or ratio of areas by the internal standardization method

y = Mean of individual values

n = Number of individual values

25) Total mobile phase time *(t_t)*

In the Size-exclusion Chromatography, the retention time of a component with molecules smaller than the smallest gel pores (refer to Figure 5).

26) Total mobile phase volume *(V_t)*

In the Size-exclusion Chromatography, the retention volume of a component with molecules are smaller than the smallest gel pores is calculated from the total mobile phase time and the flow rate *(F)* (mL/minute) using the following equation:

$$V_t = t_t \times F$$

3. SYSTEM SUITABILITY

This section applies exclusively to liquid chromatography and gas chromatography.

All components of the equipment used must be qualified and be capable of achieving the performance required to conduct the test or assay.

System suitability tests represent an integral part of the analytical procedure and are used to ensure adequate performance of the chromatographic system. Parameters such as column plate number, retention factor (mass distribution ratio), system repeatability, signal-to-noise, symmetry factor resolution and peak-to-valley ratio may be employed in assessing the performance of the chromatographic system. In cases of complex chromatographic profiles (e.g., for biotechnological/biological products), visual comparison of the profiles can be used as a system suitability test when stated in the individual monograph.

Factors that may affect the chromatographic behavior include:

- composition and temperature of the mobile phase;
- ionic strength and pH of the aqueous component of the mobile phase;
- flow rate, column dimensions, column temperature and pressure;
- stationary phase characteristics including type of chromatographic support (particle-based or monolithic), particle or pore size, porosity, specific surface area;
- reversed phase and other surface-modification of the stationary phases, the extent of chemical modification (as expressed by end-capping, carbon loading etc.).

Retention times and relative retentions may be provided in monographs for information purposes only, unless otherwise stated in the monograph. There are no acceptance criteria applied to relative retentions.

Compliance with the system suitability criteria is required throughout the chromatographic procedure. No sample analysis is acceptable unless the suitability of the system has been demonstrated.

In addition to any other system suitability criteria stated in the monograph, the following requirements must be fulfilled. When specific requirements are stated in the monograph, they supersede the requirements mentioned in this chapter:

1) System repeatability - assay of an active substance or an excipient

In an assay of an active substance or an excipient, where the target value is 100% for a pure substance, and a system repeatability requirement is not specified, calculate the maximum permitted relative standard deviation (%RSDmax) for the defined limits. This is done for a series (n = 3 to 6) of injections of the reference solution using the standard addition method. The maximum permitted relative standard deviation of the peak response does not exceed the appropriate value given in Table 1.

$$\%\mathrm{RSD}_{\max} = \frac{KB\sqrt{n}}{t_{90\%,n-1}}$$

K = Constant (0.349), obtained from the expression $K = \frac{0.6}{\sqrt{2}} \times \frac{t_{90\%,5}}{\sqrt{6}}$ in which $\frac{0.6}{\sqrt{2}}$ represents the required percentage relative standard deviation determined on 6 injections for B = 1.0;

B = Upper limit given in the definition of the individual monograph minus 100%;

n = Number of replicate injections of the reference solution ($3 \leq n \leq 6$);

$t_{90\%,n-1}$ = Student's t at the 90% probability level (double sided) with n-l degrees of freedom.

	Number of individual injections *n*			
	3	4	5	6
B (%)	*Maximum permitted relative standard deviation (%)*, $\%\mathrm{RSD}_{\max}$			
2.0	0.41	0.59	0.73	0.85
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

2) System sensitivity

The signal-to-noise ratio is used to define the system sensitivity. The limit of quantitation (corresponding to a signal-to-noise ratio of 10) is equal to or less than the reporting threshold.

To determine the signal-to-noise ratio, inject a solution of the substance to be examined at the concentration corresponding to the reporting threshold (e.g. 0.05%). Alternatively, use the reference solution used for the quantitation of unspecified impurities (e.g. 0.10% of the concentration of the test solution) and extrapolate the signal-to-noise ratio of the major peak to the reporting threshold.

3) Peak symmetry

Unless otherwise stated, in a test or assay, the symmetry factor (tailing factor) of the peak used for quantitation is 0.8 to 1.8.

4. ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions mentioned should be validated when preparing each pharmaceutical monograph. The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopoeial analytical procedures are listed below. Changes other than those indicated require revalidation of the procedure.

Multiple adjustments can have a cumulative effect on the performance of the system and are to be properly evaluated by the users. Therefore, analysts must evaluate these impacts appropriately and conduct sufficient risk assessments. This evaluation is particularly crucial when the separation pattern is presented as a profile.

Any adjustments must be made on the basis of the pharmacopoeial procedure. If adjustments are made to a pharmacopoeial procedure, additional verification tests may be required. To verify the suitability of the adjusted pharmacopoeial procedure, assess the relevant analytical performance characteristics potentially affected by the change.

If the test methods specified for pharmaceutical monographs have been adjusted in accordance with the requirements outlined below, further adjustments without proper revalidation are not permitted. Adhering to system suitability criteria is crucial to ensure that the conditions for satisfactory performance of the test or assay are met.

Adjustment of conditions with gradient elution (HPLC) or temperature programming (GC) is more critical than with isocratic (HPLC) or isothermal (GC) elution, since it may shift some peaks to a different step of the gradient or to different elution temperatures, potentially causing partial or complete co elution of adjacent peaks or peak inversion, and thus leading to the incorrect assignment of peaks, and to the masking of peaks or a shift such that elution occurs beyond the prescribed elution time.

For some parameters, the adjustments are explicitly defined in the monograph to ensure the system suitability. In the testing of biotechnological/biological products such as peptide mapping, glycosylation analysis and tests related to molecular heterogeneity, the separation pattern obtained by liquid chromatography may be as acceptance criteria in the form of a profile. In such a test method, the method shown in this section may not be applicable.

Crude drugs and related drugs are outside the scope of this section.

1) Thin-layer chromatography

Composition of the mobile phase: the amount of the minor solvent components may be adjusted by ± 30% relative or ± 2% absolute, whichever is the larger; no other component is altered by more than 10% absolute. A minor component comprises less than or equal to *(100/n)%*, *n* being the total number of components of the mobile phase. For a minor component at 10% of the

mobile phase, a 30% relative adjustment allows a range of 7 to 13% whereas a 2% absolute adjustment allows a range of 8 to 12%, the relative value therefore being the larger; for a minor component at 5% of the mobile phase, a 30% relative adjustment allows a range of 3.5 to 6.5% whereas a 2% absolute adjustment allows a range of 3 to 7%, the absolute value being the larger in this case.

pH of the aqueous component of the mobile phase: ± 0.2 pH units, unless otherwise prescribed.

Concentration of salts in the buffer component of a mobile phase: ± 10%.

Application volume: 10 to 20% of the prescribed volume if using fine particle size plates (2 to 10 µm).

2) Liquid chromatography: isocratic elution

A) Column parameters and flow rate

Stationary phase: The identity of the substituent in the stationary phase should remain unchanged (e.g., no substitution of C18 with C8). Other physico-chemical characteristics of the stationary phase, i.e. chromatographic support, surface modification and extent of chemical modification must be similar; a change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided the above-mentioned requirements are met.

Column dimensions (particle size, length): The particle size and/or length of the column may be modified provided that the ratio of the column length *(L)* to the particle size *(dp)* remains constant or in the range between - 25% to + 50% of the prescribed *L/dp* ratio.

For adjusting the particle size from totally porous to superficially porous particles, other combinations of Land *dp* can be used provided that the plate number *(N)* is within - 25% to + 50%, relative to the prescribed column.

These changes are acceptable if the system suitability criteria are fulfilled, and the selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

Column dimensions (Internal diameter): In absence of a change in particle size and/or length, the internal diameter of the column may be adjusted.

Caution is necessary when the adjustment results in smaller peak volumes. This can occur due to a smaller particle size or a smaller internal diameter, a situation which may require adjustments to minimize extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.

When changing the particle size, the flow rate must be adjusted because smaller-particle columns will require higher linear velocities to achieve the same performance (as measured by reduced plate height). The flow rate is adjusted for both the change in column diameter and particle size using the following equation:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$$

F_1 = Flow rate indicated in the monograph (mL/minute)
F_2 = Adjusted flow rate (mL/minute)
dc_1 = Internal diameter of the column indicated in the monograph (mm)
dc_2 = Internal diameter of the column used (mm)
dp_1 = Particle size indicated in the monograph (µm)
dp_2 = Particle size of the column used (µm)

When changing particle sizes from ≥ 3 µm to < 3 µm in isocratic separations, an additional increase in linear velocity (achieved by adjusting the flow rate) may be justified, provided that the column performance does not drop by more than 20%. Similarly, when a transitioning from < 3-µm to 2: 3-µm particles, an additional reduction in linear velocity (flow rate) may be justified to avoid reduction in column performance by more than 20%.

After adjustments due to changes in column dimensions, an additional change in flow rate of ± 50% is permitted.

Column temperature: ± 10 °C, unless otherwise specified in the operating temperature.

Further adjustments in analytical procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of chromatographic conditions in this chapter.

B) Mobile phase

Composition*:* The amount of the minor solvent components may be adjusted by ± 30% relative (see examples under Thin-layer chromatography); no component is altered by more than 10% absolute. A minor component comprises less than or equal to *(100/n)%*, where n is the total number of components in the mobile phase;

For a minor component at 10% of the mobile phase, a 30% relative adjustment allows a range of 7 to 13% whereas a 2% absolute adjustment allows a range of 8 to 12%, the relative value therefore being the larger. For a minor component at 5% of the mobile phase, a 30% relative adjustment allows a range of 3.5 to 6.5% whereas a 2% absolute adjustment allows a range of 3 to 7%, the absolute value being the larger in this case.

pH of the aqueous component of the mobile phase: ± 0.2 pH units, unless otherwise prescribed

Concentration of salts in the buffer component of a mobile phase: ± 10%

Flow rate: In absence of a change in column dimensions, an adjustment of the flow rate by ± 50% is permitted.

Detector wavelength: No adjustment permitted.

Injection volume: When the column dimensions are changed, the following equation may be used for adjusting the injection volume:

$$V_{inj2} = V_{inj1} (L_2 d_{c2}^2) / (L_1 d_{c1}^2)$$

V_{inj1} = Injection volume indicated in the monograph (µL)
V_{inj2} = Adjusted injection volume (µL)
L_1 = Column length indicated in the monograph (cm)
L_2 = New column length (cm)
d_{c1} = Column internal diameter indicated in the monograph (mm)
d_{c2} = New column internal diameter (mm)

This equation may not be applicable to changes from TPP columns to SPP columns.

Even in the absence of any column dimension change, the injection volume may be varied provided System Suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

3) Liquid chromatography: gradient elution

Adjustment of chromatographic conditions for gradient systems requires greater caution than for isocratic systems.

A) Column parameters and flow rate

Stationary phase: The identity of the substituent must not be changed (e.g., no substitution of C18 with C8). Other physicochemical characteristics of the stationary phase, such as chromatographic support, surface modification, and extent of chemical modification, must be similar. A change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided the abovementioned requirements are met.

Column dimensions (particle size, length): The particle size and/or length of the column may be modified provided that the ratio of the column length *(L)* to the particle size *(dp)* remains constant or in the range between - 25% to +50% of the prescribed *L/dp* ratio.

Adjustments from totally porous to superficially porous particles: Other combinations of L and *dp* can be used provided that the ratio $(t_R/w_h)^2$ is within - 25% to + 50%, relative to the prescribed column, for each peak used to check the system suitability, as stated in this chapter and the individual monograph.

These changes are acceptable if the system suitability criteria are met, and the selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

Column dimensions (internal diameter): In the absence of changes in particle size and/or length, the internal diameter of the column may be adjusted.

Caution is necessary when this adjustment results in smaller peak volumes. Adjustments may be required to minimize extra-column band broadening due to factors such as instrument connections, detector cell volume, sampling rate, and injection volume.

When the particle size is changed, the flow rate requires adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). The flow rate is adjusted for both the change in column diameter and particle size using the following equation:

$$F_2 = F_1 \times [(d_{c2}^2 \times d_{p1})/(d_{c1}^2 \times d_{p2})]$$

F_1 = Flow rate indicated in the monograph, (mL/minute)
F_2 = Adjusted flow rate (mL/minute)
dc_1 = Internal diameter of the column indicated in the monograph (mm)
dc_2 = Internal diameter of the column used (mm)
dp_1 = Particle size indicated in the monograph (μm)
dp_2 = Particle size of the column used (μm)

A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. Since the gradient volume is the gradient time, t_G, multiplied by the flow rate, *F*, the gradient time for each gradient segment needs to be adjusted to maintain a constant ratio of the gradient volume to the column volume (expressed as $L \times dc_2$). Thus, the new gradient time, t_{G2} can be calculated from the original gradient time, t_{G1}, the flow rate(s), and the column dimensions as follows:

$$t_{G2} = t_{G1} \times (F_1 / F_2) [(L_2 \times d_{c2}^2) / (L1 \times d_{c1}^2)]$$

Thus, the change in conditions for gradient elution requires three steps:

(1) adjust the column length and particle size according to *L/dp*,

(2) adjust the flow rate for changes in particle size and column diameter, and

(3) adjust the gradient time of each segment for changes in column length, diameter and flow rate. The example below illustrates this process.

Variable	Original Conditions	Adjusted Conditions	Comment
Column length *(L)* in mm	150	100	User's choice
Column diameter *(dc)* in mm	4.6	2.1	User's choice
Particle size *(dp)* in μm	5	3	User's choice
L / dp	30.0	33.3	(1)
Flow rate in mL/min	2.0	0.7	(2)
Gradient adjustment factor (t_{G2}/t_{G1})		0.4	(3)
Gradient conditions			
B (%)	Time (min)	Time (min)	
30	0	0	
30	3	(3×0.4) =1.2	
70	13	[1.2＋ (10×0.4)] =5.2	
30	16	[5.2＋ (3×0.4)] =6.4	

(1) 11% increase within allowed *L/dp* change of - 25% to + 50%
(2) *calculated using* $F_2 = F_1 [(dc_2^2 \times dp_1) / (dc_1^2 \times dp_2)]$
(3) calculated using $t_{G2} = t_{G1} \times (F_1 / F_2) [(L_2 \times dc_2^2) / (L_1 \times dc_1^2)]$

Column temperature: ± 5 ℃, where the operating temperature is specified, unless otherwise prescribed.

Further adjustments in analytical procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of Chromatographic Conditions in this chapter.

B) Mobile phase

Composition/Slope Conditions: Dwell volume composition and slope conditions can be modified under the following circumstances:

- Must meet the criteria for system suitability;
- Leak the major peaks within ±15% of the residence time obtained under the original conditions. However, this requirement does not apply when changing the column size;
- Composition and slope conditions of the dwell volume should ensure that the initial peaks are sufficiently retained, and the final peaks are eluted.

pH of the aqueous component of the mobile phase: ± 0.2 pH units, unless otherwise prescribed.

Concentration of salts in the buffer component of a mobile phase: ± 10%.

If not in compliance with the criteria for system suitability, it is advisable to consider reviewing the dwell volume or changing the column.

C) Dwell volume.

The resolution, retention time, and relative retentions may

vary significantly depending on the configuration of the equipment used. Should this occur, it may be due to a change in dwell volume. Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for analytical procedure development and that actually used. It is the responsibility of the user to adapt the isocratic step length to the analytical equipment used. If the dwell volume used during the elaboration of the monograph is given in the monograph, the time points (t min) stated in the gradient table may be replaced by adapted time points (t_c min), calculated using the following equation:

$$t_c = t - [(D - D_0) / F]$$

D = Dwell volume (mL)
D_0 = Dwell volume used for development of the analytical procedure (mL)
F = Flow rate (mL/minute)

The isocratic step introduced for this purpose may be omitted if validation data for application of the analytical procedure without this step is available.

***Detector wavelength**:* No adjustment permitted.
Injection volume: When changing column dimensions, the following equation maybe used to adjust the injection volume:

$$V_{inj2} = V_{inj1} (L_2 \, dc_2^2) / (L_1 \, dc_1^2)$$

V_{inj1} = Injection volume indicated in the monograph (μL)
V_{inj2} = Adjusted injection volume (μL)
L_1 = Column length indicated in the monograph (cm)
L_2 = New column length (cm)
dc_1 = Column internal diameter indicated in the monograph (mm)
dc_2 = New column internal diameter (mm)

This equation may not be applicable to changes from TPP columns to SPP columns.

Even without changing column dimensions, the injection volume may be varied, provided that system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.

4) Gas chromatography
A) Column parameters
Stationary phase:
— *particle size:* maximum reduction of 50%; no increase permitted (packed columns);
— *film thickness:* -50% to + 100% (capillary columns);
Column dimensions:
length: -70% to + 100%;
Internal diameter: ± 50%;

Column temperature: ± 10%;
Temperature program: Temperature adjustments are allowed as mentioned above. The adjustment of ramp rates and hold times of up to ± 20% is permitted.
Flow rate: ± 50%.

The aforementioned modifications are acceptable, provided that the system suitability criteria are met, and the selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

Injection volume and split ratio: It may be varied provided system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, or the split ratio is increased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase in injection volume or a decrease in split ratio is allowed if, specifically, the linearity and resolution of the peak(s) to be determined remain satisfactory.

Injection port temperature and transfer-line temperature in static head-space conditions: ±10 °C, provided no decomposition or condensation occurs.

5. Quantitation

The following quantitation approaches may be used in general texts or monographs:

1) External standard method

Using a calibration function: Prepare standard solutions with various concentrations of a reference standard for the target compound, ensuring a linear response range. Inject a fixed volume of these standard solutions. With the chromatograms obtained, a calibration function is prepared by plotting the peak areas or peak heights on the ordinate against the amount of reference standard on the abscissa. The calibration function is generally obtained by linear regression. Then, a test solution is prepared according to the procedure specified in the individual monograph. The chromatography is performed under the same operating conditions as for the preparation of the calibration function, the peak area or peak height of the compound to be analyzed is measured, and the amount of the compound is read out or calculated from the calibration function.

Using one-point calibration: In a pharmaceutical monograph, generally one of the standard solutions with a concentration within the linear range of the calibration function. Perform chromatography under fixed conditions, compare the obtained responses, and determine the quantity of the target compound. All procedures, such as the injection, must be conducted under constant conditions.

2) Internal standard method

Using a calibration function: In the internal standard method, a stable compound is chosen as an internal standard which shows a retention time close to that of the compound to be analyzed, and whose peak is well separated from all other peaks in the chromatogram. Several standard solutions containing a fixed amount of the internal standard and graded amounts of a reference standard of the compound to be analyzed are prepared. Calculate the ratio of the peak area or peak height of the reference standard to that of the internal standard from the chromatograms obtained. A calibration function by plotting these ratios on the ordinate against the amount of the reference standard or the ratio of the amount of reference standard to that of the internal standard on the abscissa is prepared.

The calibration function is generally obtained by linear regression. Then, prepare a test solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration function is prepared according to the procedure specified in the individual monograph. The chromatography is performed under the same operating conditions as for the preparation of the calibration function. The ratio of the peak area or peak height of the compound to be analyzed

to that of the internal standard is calculated, and the amount of the compound is read out or calculated from the calibration function.

Using one point calibration: In a pharmaceutical monograph, generally one of the standard solutions with a concentration close to that of a standard solution within the linear range of the calibration function. Include a fixed amount of the internal standard, perform chromatography under fixed conditions, and determine the quantity of the target compound by comparing the ratios obtained.

3) Normalization procedure

Provided linearity of the peaks has been demonstrated, individual monographs may prescribe that the percentage content of a component of the substance to be examined is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents or arising from the mobile phase or the sample matrix, and those at or below the disregard limit or reporting threshold.

6. Other considerations

1) Detector response

The detector sensitivity is the signal output per unit concentration or unit mass of a substance in the mobile phase entering the detector. The relative detector *response factor*, commonly referred to as response factor, expresses the sensitivity of a detector for a given substance relative to a standard substance. The *correction factor* is the reciprocal of the response factor. In tests for related substances any correction factors indicated in the monograph are applied (i.e. when the *response factor* is outside the range 0.8 to 1.2).

2) Interfering peaks

Peaks originating from solvents, reagents, the mobile phase, or the sample matrix are disregarded.

3) Measurement of peaks

The peak area of any impurity not completely separated from the major peak is preferably determined using the tangential skim method (Figure 9).

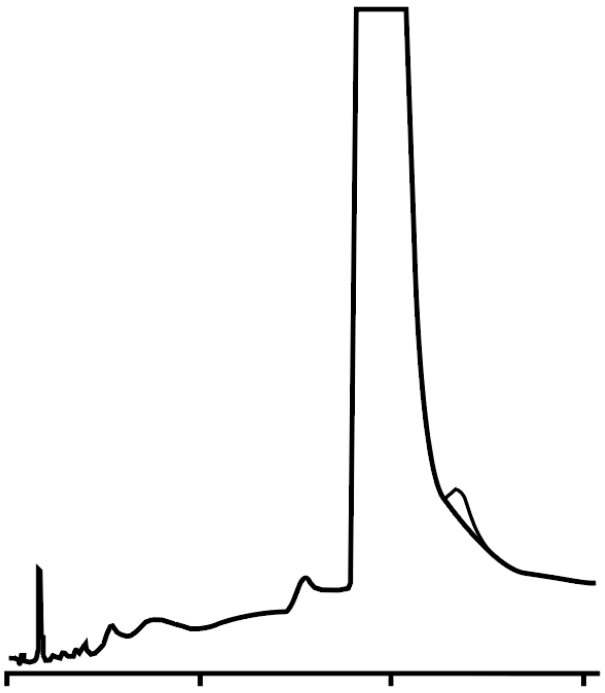


Figure 9

4) Reporting threshold

In cases where the related, it is important to choose an appropriate *reporting threshold* and appropriate conditions for the integration of the peak areas.

In such tests the reporting threshold, i.e. the limit above which a peak is reported, is generally 0.05%.

Conductivity
전도율측정법

The Conductivity is used to measure the electrical conductivity of solutions, including pure liquids. This chapter describes methods of applying conductivity to measure, monitor or control aspects such as chemical dispensing, chemical purity, ionic concentration, and other applications where the ionic characteristics of the solution are necessary. The scope of applications includes, but is not limited to, various solutions for clean-in-place, chromatography detection, preparations of ionic solutions, endpoint detection, dosing (injection of samples), fermentation and preparation of buffer solutions. For pure organic liquids, such as alcohols and glycols, which may exhibit a weak conductivity signal, the signal can be significantly increased when these organic liquids are mixed with water or salts. Therefore, conductivity can also be applied to pure organic liquids.

Conductivity measures the ability of a solution to conduct electricity via its ions. The ability of an ion to conduct electricity is directly related to its ion mobility. Conductivity has a directly proportional relationship to the concentrations of ions in the solution, which is expressed in the following equation.

$$k = 1000 \sum_{i}^{All\ ions} C_i \lambda_i$$

κ = Conductivity(S/cm)
C_i = Concentration (mol/L) of chemical ion i

λ_i = Specific molar conductance ($S \cdot cm^2/mol$) of ion *i*

Although S/m, an SI unit, is the appropriate SI unit for conductivity, historically, the unit S/cm has been the acceptable unit used by industry. According to equation (1), conductivity is not ion-selective because it responds to all ionic species. Furthermore, the specific molar conductance of each ion is different. As a result, if the percentage composition of ions in the solution is not constant or is unknown, the accurate concentrations of ionic species cannot be determined using conductivity. However, for solutions of a single salt, acid or base (e.g., an alkaline solution used in cleaning), the accurate concentration can be directly determined. Despite the lack of ionic selectivity, conductivity is an important physical quantity in laboratories or manufacturing processes for measurement and control of total ionic content because it is proportional to the sum of the concentrations of all ionic species (anions and cations) of diluted solutions, as described in equation (1). At high concentrations, conductivity measurements do not show perfect linearity with concentration. Conductivity cannot be applied to solids or gases, but can be applied to the condensate of gases.

Another factor that influences conductivity measurements is the temperature of the liquid. As the liquid temperature rises, the ion conductance increases, and this physico-chemical phenomenon is the main reason for the temperature-compensation requirement when testing conductive liquids.

The conductivity κ is proportional to the conductance (G), of a liquid between 2 electrodes, as shown in equation (2).

$$\kappa = G \times (d / A) = G \times K$$

κ = Conductivity(S/cm)
G = Conductance(S)
d = Distance between the two electrodes (cm)
A = Cross-sectional area of the electrodes (cm^2)
K = Cell constant (cm^{-1}), which is equal to the ratio of *d*/*A*.

The resistivity ρ ($\Omega \cdot cm$) of the liquid is the reciprocal of the conductivity, by definition, as shown in equation (3):

$$\rho = 1 / \kappa = 1 / (G \times K) = R / K \quad (3)$$

ρ = Resistivity ($\Omega \cdot cm$)
κ = Conductivity(S / cm)
G = Conductance(S)
K =Cell constant (cm^{-1})
R = Resistance (Ω), which is the reciprocal of Conductance (*G*).

1. Apparatus

A conductivity measurement determines the resistance of the solution between the electrodes of the cell. The basic apparatus consists of the resistance-measuring circuit and the cell for conductivity measurement. When the cell for conductivity measurement and the control unit (user interface) are separated, the resistance-measuring circuit and cell for conductivity measurement are usually connected by a cable.

The resistance is measured by applying an alternating current voltage (or alternating current; current (or voltage) in which the flow of electric charge periodically reverses direction) to the electrodes, measuring the current (or voltage), and the resistance is calculated according to Ohm's law. The alternating current source is used to prevent polarization (collection of ions) at the electrodes. The frequency of the measuring system adjusts automatically according to the measuring conditions of the apparatus, and there may be multiple resistance-measuring circuits embedded in the measuring system. The resistance-measurement circuit may be embedded in the transmitter or in the cell for conductivity measurement.

The cell for conductivity measurement consists of at least 2 electrical conductors of fixed size and geometry, separated by an electrical insulator. The electrodes, insulator and any other components in contact with liquid are composed of materials that do not react with liquids they may come into contact with. Additionally, the structure of the cell for conductivity measurement must be able to withstand the environmental conditions, including process or ambient temperature, pressure, and cleaning methods.

Many of the cells for conductivity measurement have an embedded temperature measuring device, such as a RTD or NTC thermistor. An external temperature measuring device can also be used. These temperature measuring devices are necessary for temperature compensation in conductivity measurement.

2. Cell constant determination

The cell constant of the cell for conductivity measurement is used to standardize the conductance or resistance measurements for the geometrical structure of the two electrodes.

The cell constant is determined by immersing the cell for conductivity measurement in a solution of known conductivity. Solutions of known conductivity may be obtained by preparing mixtures of solutions with specific compositions accredited by national authoritative agencies, or by purchasing commercially available certified and traceable standard solutions. The mixture of solutions or certified solutions with these compositions may range from 5 to 200, 000 µS/cm, depending on the precision required. Alternatively, the cell constant can be determined by comparison to other reference conductivity measuring systems with an accredited calibration service. (Note: conductivity values do not have a perfectly linear relationship with concentration.)

The measured cell constant of the cell for conductivity measurement must be within 5% of the nominal value indicated by the certificate of the cell for conductivity measurement, unless otherwise specified.

Modern cells for conductivity measurement generally do not need to change their cell constant over their service life. If a change in the cell constant is detected through calibration, it is necessary to clean the cell for conductivity measurement according to the manufacturer's recommendations, followed by repeating the calibration procedure. Sometimes 'memory effects' may occur, particularly when changing from high to low concentrations of solutions if the detection part is not properly cleaned.

3. Temperature calibration

In addition to testing the cell constant of the cell for conductivity measurement, the embedded temperature measuring device (or external temperature measuring device) must be appropriately calibrated to ensure the accurate application of temperature compensation. The required temperature accuracy depends on the importance of the temperature to the application, but in general, ±1 ℃ would suffice.

4. Calibration of measurement electronics

The measurement circuit of the system is essentially an alternating current (AC) resistance meter. Appropriate testing or calibration is necessary for measurement systems performing signal transfer via analog cables. This is performed by disconnecting the measuring circuit from the cell for conductivity measurement,

attaching traceable resistors with a known resistance value to the measuring circuit using the same measurement system cable, and verifying that the measured resistance meets the specified resistance value. A typical acceptance criterion for the accuracy of the resistance value is less than 2% for resistances greater than 100 Ω, and increases to 5% at lower resistances. However, the precise acceptance criterion is ultimately determined based on the required accuracy of the measurements.

For equipment that cannot have the resistance-measuring circuit disconnected from the electrodes (e.g. measuring circuit and electrodes are integrated), it may be difficult to directly adjust or verify the circuit accuracy, depending on the structure of the cell for conductivity measurement. An alternative method of testing the integrity of the measurement system is to perform system calibration according to the procedures for cell constant determination for each measuring circuit to be used.

When testing/calibrating the cell constant of the cell for conductivity measurement, temperature measuring device and measuring circuit is conducted in the same manner on a regular basis, the measuring circuit is tested first, followed by the temperature measuring device and the cell constant. Because all of these parameters are stable in modern electronics or stable sensor instrumentation, frequent calibration is not required. Comparison to qualified reference systems is also one of the methods of calibration. Calibration is performed at appropriate intervals as prescribed in the quality management system.

5. Temperature compensation

As the conductivity of a liquid is temperature-dependent, temperature compensation of the conductivity measurement is usually necessary unless otherwise specified (e.g. purified water, water for injection). An appropriate temperature compensation algorithm will ensure that changes in the conductivity measurements can be attributed to concentration changes and not temperature changes. Conductivity measurements are typically conducted with 25 °C as the reference temperature. A general form of linear temperature compensation is represented in equation (4).

$$\kappa_{25} = \kappa_T / (1 + \alpha(T\text{-}25)) \quad (4)$$

κ_{25} = Conductivity compensated to 25 °C
κ_T = Conductivity at T °C
α = Temperature coefficient of conductivity
T = Measured temperature

A temperature coefficient of 2.1%/°C is commonly used for most salt solutions. Most salt solutions have linear temperature coefficients of conductivity ranging from 1.9 to 2.2%/°C. Depending on the liquid samples, other types of temperature compensation may be appropriate. Non-linear temperature compensation is performed using pre-programmed data in the device. Non-linear temperature compensation data for various solutions can be widely used for natural water or ultra-pure water containing trace amount of ammonia.

In cases of very low conductivity (below 10 μS/cm), such as purified water for the cleaning or rinsing of instruments, double temperature compensation is required. One is for the intrinsic conductivity of water, and the other is for the other ionic species detected in water. These compensations are usually interlinked in microprocessor-controlled conductivity measurement systems. However, this function is not provided in all conductivity measurement technologies.

6. Conductivity measurement of solutions

For off-line or at-line batch measurements, rinse the cleaned cell for conductivity measurement with the solution to be measured. Then, immerse the cell for conductivity measurement in the solution to be measured and record the temperature and temperature-compensated conductivity. Ensure that the position of the sensor in the container does not impact the conductivity measurement, as the container walls may impact the measurement depending on the electrode design.

For continuous on-line or in-line measurements, install the cleaned cell for conductivity measurement into the pipe, tank or other well-closed containers, and clean if necessary. Make sure that proper installation procedures are applied to prevent bubbles or foreign matter from entering between the electrodes. Ensure that the position of the cell for conductivity measurement in the pipe or tank does not impact the conductivity measurement, as their surfaces may affect the measurement depending on the electrode design. Record the temperature and the temperature-compensated conductivity.

For all batch or continuous measurements, ensure that the immersed components of the cell for conductivity measurement are compatible with the solution to be measured and the temperature conditions.

Congealing Temperature
응고점측정법

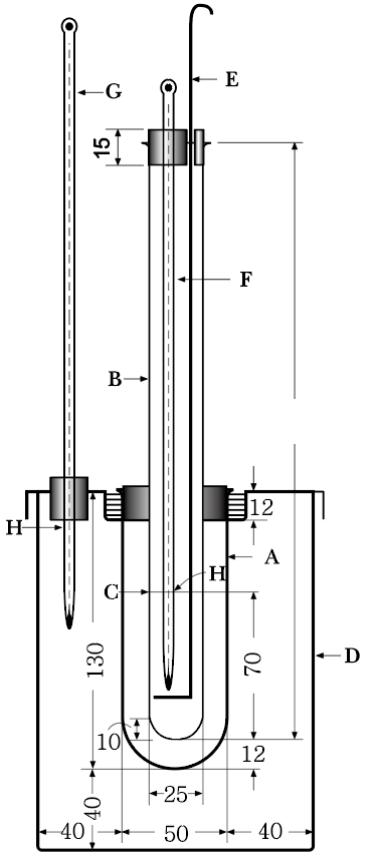
The congealing temperature is determined by the following method.

Apparatus

Use the apparatus shown in the figure.

Procedure

Fill the sample in the container B up to the gauge line C. If the sample is solid, melt it by heating, ensuring the temperature does not exceed 20 °C above the anticipated congealing temperature, before transferring it to container B. Container D, made of glass or plastic, is filled almost completely with water approximately at a temperature 5 °C below the anticipated congealing temperature. For samples that are liquid at ordinary temperature, fill bath D with water at 10 °C to 15 °C below the expected congealing temperature. Place the sample-filled container B inside cylinder A. Align the immersion line H of thermometer F with the sample's meniscus. Cool the sample until it is roughly 5 °C above the expected congealing temperature. Then, using the stirrer E, stir vertically at a pace of 60 to 80 strokes per minute. Monitor the thermometer readings every 30 seconds. As the temperature slowly drops and an appreciable quantity of crystals forms, stop stirring when the temperature stabilizes or begins to increase. Usually, after the temperature rises, the highest temperature (as for thermometer F) maintained for a while is read. If no temperature increase is observed, note the steady temperature. If the mean of at least four consecutive temperature readings falls within a 0.2 °C, consider this as the congealing temperature.



* The figures are in mm.

Figure

A: Glass cylinder (Both sides of the wall are coated with silicone oil to prevent clouding)
B: Sample container (A hard glass test tube. All areas, except where in contact with the sample, are coated with silicone oil to prevent clouding. Insert into cylinder A and fix with a cork stopper)
C: Gauge line
D: Bath made of glass or plastic
E: Stirring rod, either glass or stainless steel (3 mm diameter; the lower end forms a loop approximately 18 mm in diameter)
F: Thermometer with an immersion line
G: Thermometer (Either with an immersion line or a total immersion type)
H: Immersion line

Note: When supercooling is expected, gently rub the inner wall of bath B or introduce a tiny piece of the solid sample into bath B. This encourages congealment as the temperature nears the anticipated congealing temperature.

Crude Drugs Test
생약시험법

The Crude Drugs Test is applied to all crude drugs and their preparations in the monograph.

Sampling

Unless otherwise specified, samples are collected according to the following method and if necessary, preserve them in an airtight container.

A. For small-sized, cut, and powdered crude drugs, mix well and collect 50 - 250 g of sample.

B. For big-sized crude drugs, mix well by stirring and collect 250 to 500 g of sample.

C. For crude drugs weighing 100 g or more, collect five pieces or more as sample or cut them into appropriate sizes, mix well by stirring, and then collect 500 g or more.

Preparation of the sample for analysis

Mix the sample well and use powdered crude drugs as they are for analysis. For non-powdered drugs, powder them unless otherwise specified. For drugs that cannot be powdered, cut them as small as possible, spread them thinly, and use the evenly distributed part as a sample for analysis. If necessary, preserve it in an airtight container.

Microscopic examination

A. Equipment

Use an optical microscope. Use 10x, and 40x object lenses and a 10x eyepiece.

B. Preparation of the sample

1) Section

Place a section on a slide glass and add 1 - 2 drops of a mounting agent. Gently place a cover glass on top while avoiding trapping any air bubbles. The thickness of the section used for observation is usually 10 - 20 μm.

2) Powder

Place 1 mg of powdered sample on a watch glass and add 1 - 2 drops of swelling agent. Mix well with the tip of a small glass rod, while avoiding trapping any air bubbles and then allow to stand for 10 minutes to swell the sample. Smear a small amount of the swollen sample onto a slide glass with the tip of a glass rod, and add a drop of a mounting agent. Spread it evenly to avoid particles of tissue from overlapping, and then place a cover glass gently on top, while avoiding trapping any air bubbles.

Unless otherwise specified, use glycerin and water (1:1) mixture; or glycerin, 95 vol% ethanol and water (1:1:1) mixture for mounting and swelling agents.

C. Observation of each element of the description section

In accordance with the listed order in the monograph, sections should be observed, in order; exterior, interior, and cell inclusions. Powders should be observed in the order of special characteristics, commonly occurring characteristics, rarely occurring characteristics and cell inclusions.

Purity

A. Foreign matter

Unless otherwise specified, weigh 25 to 500 g of sample, spread it evenly, and eliminate any foreign matter identified by the naked eye or through a 10x magnifying glass. Weigh the mass of the foreign matter to calculate its percentage.

B. Heavy metal

1) Lead, Arsenic, Cadmium

A) Preparation of the test solution

① Microwave decomposition method: Powder a certain amount of the sample for analysis, weigh accurately 0.1 to 0.5 g, place it into a microwave sample pre-treatment container, and

add 12 mL of nitric acid. If the sample does not decompose well, add 1 to 2 mL of hydrochloric acid or hydrogen peroxide (30). Remove the generated gas by placing the container in the hood and break it down further using the microwave sample pre-treatment apparatus. When it is completely decomposed, filter the liquid into a volumetric flask using a filter paper, add water and dilute appropriately to the concentration range of the standard solution, and use it as the test solution. Separately, place 12 mL of nitric acid into the microwave sample pre-treatment container, repeat the same procedure of the test solution, and use it as blank test solution.

② Wet decomposition method: Powder a certain amount of sample for analysis, weigh accurately 2 to 5 g, place it into a beaker and add 10 to 30 mL of nitric acid. Cover it with watch glass and allow to stand overnight. Place it on a heating plate and slowly increase the temperature to decompose until no brown smoke is generated. If it does not decompose, add additionally 10 mL of nitric acid. Add 5 - 10 mL of hydrogen peroxide (30) to decompose it completely until it turns light yellow or yellow. If it does not decompose completely, microwave sample pre-treatment apparatus can be used. Concentrate this solution on a heating plate until it reaches 1 to 2 mL. After cooling, filter the liquid into a volumetric flask using filter paper, add water and dilute appropriately to the concentration range of the standard solution. Use it as the test solution.

In the case of arsenic, add 1 mL of potassium iodide test solution to 10 mL of the test solution and then dilute to exactly 50 mL with 0.5 mol/L of hydrochloric acid to use the test solution for measuring arsenic (skip this process when measuring with ICP-MS).

Separately, repeat the same procedure of the test solution with 10 to 30 mL of nitric acid, and use it as blank test solution.

B) Measurement

Measurement can be done using the test solution, standard solution and blank test solution by following the Atomic Absorption Spectrometric method. Using the Atomic Absorption Spectrometry, dilute appropriately 1000 mg/L of the standard solution used for atomic absorption analysis for each heavy metal with 0.5 mol/L nitric acid and prepare a calibration curve. Calculate the absorbance (or intensity) of the test solution by correcting it with a blank test solution.

Inductively Coupled Plasma Spectrometer (ICP), or Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), can be used instead of Atomic Absorption Spectrometry (AAS) for measurement.

2) Mercury

Powder a certain amount of the sample for analysis, weigh accurately 10 to 500 g, and measure using a mercury analyzer.

3) Heavy metal

Weigh 1 g (or 1 mL) of the sample and proceed with the test following the third method of the Heavy Metals. Add 3.0 mL of the lead standard solution to the control solution.

4) Determination

Follow the relevant regulations specified in the monograph. Unless otherwise specified, follow the regulations below:

A) Plant derived crude drugs: contain less than 5 ppm of lead; less than 3 ppm of arsenic; less than 0.2 ppm of mercury; and less than 0.3 ppm of cadmium

B) Animal derived crude drugs: contain less than 30 ppm of heavy metals

C) Extract of crude drugs: contain less than 30 ppm of heavy metals

D) Medicines made only from crude drugs: contain less than 30 ppm of heavy metals; less than 5 ppm of lead; and less than 3 ppm of arsenic

C. Pesticide residues

1) Napropamide, DDT (p,p'-DDD, p,p'-DDE, o,p'-DDT and p,p'-DDT), Dieldrin, Myclobutanil, Methyl-pentachlorophenyl sulfide, Methoxychlor, BHC (α, β, γ, and δ-BHC), Bifenthrin, Cypermethrin, Cyprodinil, Acetamiprid, Azoxystrobin, Aldrin, Endosulfan (α, β-Endosulfan and Endosulfan sulfate), Endrin, Chinomethionate, Cadusafos, Captan, Quintozene (PCNB), Kresoxim-methyl, Chlorothalonil, Chlorpyrifos, Chlorfenapyr, Tebuconazole, Tebufenpyrad, Terbufos, Tetradifon, Tolylfluanid, Triadimenol, Triadimefon, Triflumizole, Thifluzamide, Fenarimol, Pendimethalin, Pentachloroaniline, Fenpropathrin, Fosthiazate, Procymidone, Prochloraz, Fludioxonil, Pyrimethanil, Hexaconazole

A) Preparation of the test solution

① Extraction: Powder 500 to 600 g of this medicine well, mix 5g of it with 40 mL of water, and allow to stand for 4 hours (the amount of the sample can be adjusted if necessary). Add 90 mL of acetone to the mixture, blend it for 5 minutes using a homogenizer, and then filter it under reduced pressure using a vacuum pump, a Erlenmeyer flask with branch and a Buchner funnel. Transfer this filtered solution into a 500 mL separatory funnel and add 50 mL of a saturated sodium chloride solution and 100 mL of water. Add 70 mL of dichloromethane to the mixture, shake it vigorously, and then allow to stand to separate the layers. Take the bottom layer (dichloromethane layer) and put it in another separating funnel. After adding 70 mL of dichloromethane to the water layer and shaking vigorously, allow to stand to separate the layers, and then take the bottom layer (dichloromethane layer). Dry the dichloromethane layer by passing anhydrous sodium sulphate through it, concentrate it under reduced pressure and then dissolve it in 4 mL of hexane.

② Purification: Add 6 mL hexane into a Florisil cartridge [cartridge filled with Florisil (1 g), volume 6 mL] and elute it after 2 minutes. Pour 6 mL of hexane-acetone mixture (4:1) into the cartridge and elute again in the same way. Then, pour the extracted solution into the top end of the cartridge and slowly collect the effluent after 2 minutes. While the cartridge is still wet with the solvent, elute 5mL of hexane-dichloromethane-acetone mixture (100:97:3) (hexane-acetone mixture (7:3) in the case of prochloraz and thifluzamide), and collect the effluent. Concentrate the effluent under reduced pressure on a water bath at below 40 °C and make the test solution by dissolving it in 2 mL of acetone-hexane mixture (1 in 5).

B) Preparation of the standard solution

① Undiluted standard solution: Dissolve each standard pesticide in acetone, etc. to make 100 ppm.

② Standard solution: Mix and dilute each standard stock solution to appropriate concentration using acetone.

C) Reagents and test solutions

① Water: distilled water or equivalent

② Solvent: solvents for testing agrochemical residues or equivalent

③ Other reagents: reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard so-

lution following the gas chromatography under the conditions below:

Operating conditions

ⓐ Detector: Electron Capture Detector (ECD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 5% methyl silicone for gas chromatography in 0.25 μm thickness, a silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 50% phenyl and 50% methyl silicone for gas chromatography in 0.25 μm thickness, or equivalent.

Column temperature: Inject the sample into the column at 80 °C. After 2 minutes, increase temperature by 10 °C every minute until 280 °C, and allow to stand for more than 10 minutes (over 15 minutes if the column has been coated with 50% phenyl and 50% methyl silicone in 0.25 μm thickness).

Sample injection port temperature: 260 °C
Detector temperature: 280 °C
Carrier gas: Nitrogen
Flow rate: 1.0 mL/min
Split ratio: 10:1

ⓑ Detector: Nitrogen-Phosphorus Detector (NPD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 5% methyl silicone for gas chromatography in 0.25 μm thickness, a silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 50% phenyl and 50% methyl silicone for gas chromatography in 0.25 μm thickness, or equivalent.

Column temperature: Inject the sample into the column at 80 °C. After 2 minutes, increase temperature by 10 °C every minute until it reaches 280 °C, and allow to stand for 10 minutes (15 minutes if the column has been coated with 50% phenyl and 50% methyl silicone in 0.25 μm thickness).

Sample introduction port temperature: 260 °C
Detector temperature: 280 °C
Carrier gas: Nitrogen
Flow rate: 1.0 mL/min
Split ratio: 10 ：1

ⓒ Detector: Mass spectrometer Detector (MSD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m, used for MSD) coated with 5% methyl silicone for gas chromatography in 0.25 μm thickness, or equivalent.

Column temperature: Inject sample into the column at 100 °C. After 2 minutes, increase temperature by 10 °C every minute until it reaches 280 °C, and allow to stand for more than 15 minutes.

Sample injection port temperature: 260 °C
Interface temperature: 280 °C
Carrier gas: Helium
Flow rate: 0.9 mL/min
Split ratio: 10 ： 1

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

2) Metiram, thiram and propineb

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well, weigh 20 g accurately, and place it into an Erlenmeyer flask. Add 0.5 g of L-cysteine hydrochloride to 80 mL of 0.45 mol/L sodium hydroxide solution (adjust pH to 9.5 - 9.6, precisely) containing 0.25 mol/L ethylenediaminetetraacetic acid disodium salt. Immediately cover it with a stopper and shake it for 10 minutes. Filter this solution using a glass filter. Rinse the Erlenmeyer flask and residues with 10 mL of the above extracted solvent several times, and then add it to the filtered solution. Add 5 mL of 0.41 mol/L tetrabutylammonium hydrogen sulfate aqueous solution and 10 g of sodium chloride, and mix them well by shaking. Immediately adjust the pH to 7.0 using 2 mol/L hydrochloric acid TS, and transfer this solution to a 300 mL separatory funnel.

Note: When the sample is homogenized through grinding or pulverizing, the dithiocarbamate family agrochemicals are decomposed rapidly. As these agrochemicals are unstable in alkaline conditions and decomposes immediately after extraction using the extracted solvent, the extraction should be limited to 15 minutes, rinsing and filtering time should be minimized, and the pH should be adjusted to 7.0 immediately.

② Derivatization: After placing 40 mL of dichloromethane-hexane mixture (1:1) containing 0.05 mol/L iodomethane into the above separatory funnel, shake it vigorously for 5 minutes and allow to stand. After transferring the organic solvent layer (the top layer) to a 50 mL centrifuge tube, centrifuge it for 5 minutes at 800 rpm and take 20 mL from the tube. Mix it with 5 mL of a dichloromethane solution (1 in 5) containing 1,2-propanediol, and solvents except 1,2-propanediol are removed under reduced pressure on a water bath at 30 °C or less under a nitrogen stream. Immediately dissolve it in methanol, and use a certain amount of this solution as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard thiram in methanol. Completely dissolve standard metiram and standard propineb in a sample extraction solvent [0.5 g of L-cysteine hydrochloride hydrate mixture and 100 mL of 0.45 mol/L sodium hydroxide solution containing 0.25 mol/L ethylenediaminetetraacetic acid disodium salt (adjust the pH to 7.0 using hydrochloric acid)] to make 100 mg/L concentration and use it immediately.

② Standard solution: Dilute the above standard stock solution to an appropriate concentration with an extracted solvent adjusted to pH 7.0. Make sure that 1 mL of the solution is operated under the same process of A) Preparation of the test solution, ① Extraction and ② Derivatization, and dilute it to an appropriate concentration and use.

C) Reagents and test solutions

① Water: distilled water or equivalent

② Solvent: solvents for testing agrochemical residues or equivalent

③ Other reagents: reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 272 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 to 5 mm and length 20 to 30 cm).

Mobile phase: water, acetonitrile and methanol (65:22:13) mixture

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC-MS/MS, the components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test. It is detected in the order of thiram, metiram, and propineb.

3) Azocyclotin

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug, weigh about 30 g, and mix it with 40 mL of water and 10 mL of acetic acid well. Add 100 mL of acetone to it, homogenize for 3 minutes using a homogenizer, and filter it under reduced pressure. Rinse the residue with a small amount of acetone. Collect the filtered solution, transfer it to a separatory funnel, and extract it twice using 100 mL of hexane. Remove water using anhydrous sodium sulfate and concentrate the solution under reduced pressure.

② Derivatization: After dissolving the concentrated solution in 20 mL of ether, add 3 mL of 3 mol/L magnesium chloride-tetrahydrofuran solution, shake the mixture well, and then allow to stand for 10 minutes. Add 10 mL of water and 1 mL of hydrochloric acid to hydrolyze, and then transfer it to a separatory funnel. Rinse the flask thoroughly with 10 mL ether and collect the solution in the separatory funnel. After discarding the aqueous layer (the bottom layer) and collecting the ether layer, remove water using anhydrous sodium sulphate and concentrate to dry it.

③ Purification: Add hexane to 10 g of Florisil [Heat Florisil (60 to 100 mesh) for column chromatography overnight at 130 ℃, then cool in a desiccator] in a glass column (internal diameter 20 mm and length 30 mm). After dissolving the concentrated solution in about 10 mL of hexane, transfer the solution to the column prepared in advance, elute 120 mL of hexane and collect the effluent. Concentrate and dry the effluent under reduced pressure on a water bath at below 40 °C, dissolve it in acetone, and use a certain amount of this solution as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard azocyclotin in hexane to make 100 ppm.

② Standard solution: Dilute the standard stock solution with hexane to an appropriate concentration.

C) Reagents and test solutions

① Water: distilled water or equivalent

② Solvent: solvents for testing agrochemical residues or equivalent

③ Other reagents: reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution following the gas chromatography under the conditions below:

Operating conditions

Detector: Flame photometric detector (FPD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 50% cyanopropphenyl and 50% methyl silicone and for gas chromatography at a thickness 0.25 μm, or equivalent.

Column temperature: Inject the sample into column at 80 °C and maintain this temperature for 2 minutes. Then increase temperature by 10 ℃ every minute until 260 °C, and allow to stand for over 20 minutes.

Sample injection port temperature: 270 ℃

Detector temperature: 300 ℃

Carrier gases and flow rate: Nitrogen (1.0 mL/min), hydrogen (3 mL/min), air (30 mL/min)

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

4) Carbendazim

A) Preparation of the test solution

① Extraction (1st): Powder 500 - 600 g of this drug well, mix 10 g of it with 4 g of sodium L-ascorbate, 40 mL of water, 80 mL of methanol and 5 g of hyflosupercell, and extract by shaking the mixture for 1 hour. Filter it under reduced pressure. Rinse the container and residues using 50 mL of methanol and add it to the filtered solution.

② Extraction (2nd): Transfer the extracted solution to a separatory funnel, add 200 mL of water, 20 mL of a saturated solution of sodium chloride, and adjust the pH to 2 - 3 using diluted hydrochloric acid. Extract the solution twice using 70 mL of hexane and then discard the hexane layer. After adjusting the pH of the water layer to 6 - 7, extract the solution of the aqueous layer twice using 100 mL of ethyl acetate. Filter the solution through 1 PS filter paper, concentrate and dry the filtered solvent layer on a water bath at below 40 °C.

③ Purification: Fill 5 g of Florisil [Heat Florisil for column chromatography overnight at 130 °C, then cool in a desiccator] with hexane in a glass column (internal diameter 15 mm and length 300 mm). Transfer the concentrated residue into 5 mL of a hexane and acetone (7 ：3) mixture, elute 80 mL of a hexane and acetone (7 ：3) mixture, and collect the effluent. Concentrate and dry the effluent on a water bath at below 40 °C, dissolve it in 2 mL of methanol, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard carbendazim in methanol to make 100 ppm.

② Standard solution: Dilute the standard stock solution with methanol to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 285 nm), Fluorescence photometer (excitation wavelength, 280 nm; fluorescence wavelength, 315 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 - 5 mm and length 20 - 30 cm).

Column temperature: Constant temperature around 40 °C

Mobile phase: 0.01 mol/L potassium dihydrogen phosphate TS and methanol mixture (3:2)

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

5) Difenoconazole

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix about 50 g of it with 100 mL of acetone, and extract by shaking it for 30 minutes. Filter by passing through a filter aid (Celite 545) under reduced pressure, add 50 mL of a saturated solution of sodium chloride, and extract the solution twice using 50 mL of hexane. Remove water by passing an anhydrous sodium sulfate through hexane layer, concentrate under reduced pressure on a water bath at below 40 °C, and dissolve it in 5mL of hexane.

② Purification: In advance, elute 5 mL of hexane into the Florisil cartridge [cartridge filled with Florisil (1 g), capacity 6 mL] at a rate of 2 to 3 drops per second and discard. Make sure that the cartridge absorbs the extracted solution. Elute 20 mL of hexane-acetone mixture (19:1) and discard the effluent. Elute 40 mL of hexane-acetone mixture (7:3) and collect the effluent. Concentrate the effluent under reduced pressure on a water bath at below 40 °C, dissolve it in 2 mL of acetone, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard difenoconazole in acetone to make 100 ppm.

② Standard solution: Dilute the standard stock solution using acetone to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution following the gas chromatography under the conditions below:

Operating conditions

Detector: Nitrogen-Phosphorus Detector (NPD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm, and length 30 m) coated with 50% phenyl and 50% methyl silicone for gas chromatography in 0.25 μm thickness, or equivalent.

Column temperature: Inject the sample into the column at 100 °C. After 1 minute, increase temperature by 10 °C every minute until 250 °C, and allow to stand for over 12 minutes.

Sample injection port and detector temperature: 280 °C

Carrier gas: Nitrogen

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

6) Imidacloprid

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well, weigh about 25 g of it and mix it with 100 mL of acetonitrile and 100 mL of water accurately. Homogenize the mixture for 5 minutes at a high speed. After filtering the extracted solution under reduced pressure, collect accurately 100 mL of the filtered solution. Concentrate this solution under reduced pressure on a water bath at below 40 °C until only water is left. The water layer is extracted twice using 50 mL of cyclohexane, and the cyclohexane layer is discarded and the solution is extracted twice using 50 mL of dichloromethane. Remove water by passing an anhydrous sodium sulfate through the extract, concentrate it under reduced pressure on a water bath at below 40 °C, and dissolve it in 2 mL of dichloromethane.

② Purification: After adsorbing the extract on a silica gel cartridge activated in advance [cartridge filled with silica gel (1 g) for SPE (capacity, 6 mL) or equivalent], elute 10 mL of a hexane and ethyl acetate (1:1) mixture and discard, and then elute with 15 mL of ethyl acetate and hexane (7:3) mixture. Concentrate and dry the solution, dissolve it in an acetonitrile-water mixture (1:1), and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard imidacloprid in water-acetonitrile mixture (4:1) to make 100 ppm.

② Standard solution: Dilute the standard stock solution with water-acetonitrile mixture (4:1) to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 270 nm).

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 - 5 mm and length 20 - 30 cm).

Column temperature: Constant temperature around 40 °C.

Mobile phase: A mixture of 0.01 mol/L disodium hydrogen phosphate (pH 6.5) and acetonitrile (3:1).

Flow rate: 0.8 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

7) Iminoctadine

A) Preparation of the test solution

Powder 500 - 600 g of this drug well and mix 20 g of it well with 7g of guanidine hydrochloride. Add accurately 100 mL of methanol to the solution and homogenize it for about 5 minutes at a high speed. After filtering the extracted solution under reduced pressure, take accurately 50 mL of it. Add 50 mL of 2 mol/L sodium hydroxide TS, 50 mL of water and 100 mL of chloroform to the 50 mL of the filtered solution and distribute the mixture 3 times for 2 minutes to obtain the organic solvent layer. Add 2 mL of 1 mol/L sulfuric acid test solution and 40 mL of water, distribute the mixture to obtain the water layer. Add 0.5 mL of 1 mol/L sulfuric acid test solution and 20 mL of water again. After mixing the water layers, concentrate it on a water bath at about 70 °C to 2 mL, add sodium acetate aqueous solution (4 in 25) to the concentrated solution to make 5 mL and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve iminoctadine triacetate in water to make 100 ppm iminoctadine (Iminoctadine scale factor: 0.664).

② Standard solution: Dilute the standard stock solution with water to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Fluorescence photometer (excitation wavelength, 305 nm; fluorescence wavelength, 500 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 - 5 mm and length 20 - 30 cm).

Column temperature: Constant temperature around 40 °C

Mobile phase: After changing the concentration ratio from A:B mixture (8:2) to A : B mixture (1:9) for 30 minutes, let it flow for 10 minutes (or other optimum conditions).

A - water and ammonia (28) (69:1) mixture (Adjust pH to 2.5 using 60% perchloric acid.)

B - Diluted methanol (2 in 5)

Flow rate: 0.7 mL/min

Post-column derivatization reaction pump flow rate: 0.7 mL/min. (0.5 mol/L sodium hydroxide solution, 0.7 mL/min, and ninhydrin solution (3 in 20), 0.7 mL/min.) (or other optimum conditions)

Reactor temperature: 80 °C

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

8) Pymetrozine

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g of it with 100 mL of methanol and 30 mL of water. Leave the mixture at ordinary temperature for 2 hours and then extract it by shaking for 1 hour. After filtering the extracted solution under reduced pressure using a filter aid (Celite® 545), rinse off the residues with 100 mL of methanol and mix it with the filtered solution to make exactly 250 mL. Accurately take 100 mL of the solution, put it in a separatory funnel, and add 100 mL of hexane. Shake the mixture and then discard the hexane layer. Collect the methanol layer and concentrate it under reduced pressure until about 5 mL is left.

② Purification: After making sure that the extracted solution is absorbed into a graphitized carbon cartridge pre-activated with 5 mL of methanol and 5 mL of water (cartridge filled with 250 mg of graphitized carbon, capacity 6 mL), elute a 5 mL methanol and water (1:1) mixture, a 5 mL methanol and acetonitrile (7:3) mixture and a 5mL ethyl acetate mixture to remove impurities. Dry it under reduced pressure for 3 minutes, elute 30 mL of

dichloromethane, and collect the effluent. After concentrating and drying the effluent under reduced pressure in water bath at below 35 °C, dissolve it in 2 mL of acetonitrile, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard pymetrozine in acetonitrile to make 500 ppm.

② Standard solution: Dilute the standard stock solution with acetonitrile to an appropriate concentration

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 300 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 - 5 mm and length 20 - 30 cm).

Mobile phase: water and acetonitrile (87:13) mixture

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

9) Thiamethoxam

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g of it with a 100 mL ethyl acetate and acetone (3:2) mixture. Shake the mixture for 30 minutes. Pass the extracted solution through a filter aid (Celite® 545) and remove the solvent by concentrating it under reduced pressure. Add 50 mL of sodium chloride test solution and extract the solution twice by adding 50 mL of dichloromethane each time. Remove water by passing an anhydrous sodium sulfate through the extracted solution. After concentrating and drying under reduced pressure, dissolve it in a 5 mL of hexane-acetone mixture (9:1).

② Purification: Make sure that the extracted solution is absorbed into a silica gel cartridge [cartridge filled with silica gel (1 g), capacity 6 mL], and elute 10 mL of hexane and acetone (9∶1) mixture and discard. Elute a 20 mL hexane and acetone (3:2) mixture and then collect the effluent. Concentrate and dry the effluent under reduced pressure, dissolve it in 2 mL of acetonitrile, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard thiamethoxam in acetonitrile to make 100 ppm.

② Standard solution: Dilute the standard solution with acetonitrile to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 300 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 2 - 5 mm and length 20 - 30 cm).

Column temperature: Constant temperature around 25 °C

Mobile phase: water and acetonitrile (1:1) mixture

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

10) Triforine

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g of it with 200 mL of acetone. Homogenize and filter the mixture under reduced pressure. After removing acetone and transferring it to a separatory funnel, add 200 mL of sodium chloride solution (1 in 20) and extract the solution twice using 100 mL of benzene each time. Collect the benzene layer, concentrate it under reduced pressure, and dissolve it in 5 mL of acetone.

② Purification: Fill 5 g with silica gel [silica gel for column chromatography (70 ~ 230 mesh)] activated overnight at 130 °C and 2 g of anhydrous sodium sulfate into a glass column (internal diameter 10 mm and length 40 mm), and elute 50 mL of hexane and discard the effluent. Dissolve the concentrated solution in a small amount of benzene and make sure that the column absorbs it. Elute 150 mL of hexane and acetone (9:1) mixture, and discard the effluent. Effuse 100 mL of the hexane and acetone (7:3) mixture again, and collect the effluent. Concentrate and dry the effluent under reduced pressure, dissolve it in a methanol and ethyl acetate (1:1) mixture, and use a certain amount of it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard triforine in acetone to make 100 ppm.

② Standard solution: Dilute the standard stock solution using acetone to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution following the gas chromatography under the conditions below:

Operating conditions

Detector: Electron Capture Detector (ECD)

Column: A silica glass capillary column tube (internal diameter 0.25 mm and length 30 m) coated with 50% phenyl and 50% methyl silicone for gas chromatography in 0.25 μm thickness, or equivalent.

Column temperature: 150 °C

Sample injection port temperature: 270 °C

Detector temperature: 280 °C

Carrier gas: Nitrogen

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

11) Dithianon

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g ot it with 5 mL of 4 mol/L hydrochloric acid and 100 mL of acetone. Shake the mixture for 30 minutes. Filter the extracted solution under reduced pressure using a filter aid (Celite® 545), a vacuum pump, a Erlenmeyer flask with branch and a Buchner funnel. After removing acetone by concentrating the extracted solution under reduced pressure on a water bath at below 40 °C, transfer it to a separatory funnel by using 50 mL of sodium chloride solution (1 in 20) and 50 mL of hexane. Shake the solution vigorously and allow to stand to separate the layers. Transfer the bottom layer (water layer) to another separatory funnel, and add 50 mL of hexane. Shake the solution vigorously and allow to stand to separate the layers. Collect the hexane layer and remove water by passing an anhydrous sodium sulfate. Concentrate it under reduced pressure and then dissolve it in 5 mL of dichloromethane.

② Purification: Fill 5g of silica gel (60 - 100 mesh) and 2g of anhydrous sodium into a glass column (internal diameter 14 mm and length 400 mm), elute 50 mL of hexane, and discard the effluent. Make sure that 5mL of the extracted solution is absorbed. After effusing 50 mL of benzene and concentrating the effluent under reduced pressure, dissolve it in 1 mL of acetonitrile, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard dithianon in an acetone solution containing acetic acid (1 in 20) to make 100 ppm.

② Standard solution: Dilute the standard stock solution with an acetone solution containing acetic acid (1 in 20) to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 254 nm).

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 4.6 mm and length 25 cm).

Column temperature: Ordinary temperature (30 °C)

Mobile phase: methanol, water and acetonitrile (70:30:1) mixture

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

12) Fenpyroximate

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g of it with 100 mL of acetone (7 in 10). Shake the mixture for 30 minutes. Filter the extracted solution under reduced pressure using a filter aid (Celite® 545), a vacuum pump, an Erlenmeyer flask with branch and a Buchner funnel. After removing acetone by concentrating the extracted solution under reduced pressure on a water bath at below 40 °C, transfer it to a separatory funnel by using 50 mL of sodium chloride solution (1 in 20) and 50 mL of hexane. Shake the solution vigorously and allow to stand to separate the layers. Transfer the bottom layer (water layer) to another separatory funnel, and add 50 mL of hexane. Shake the solution vigorously and allow to stand to separate the layers. Collect all of the hexane layers and remove water by passing an anhydrous sodium sulfate through the layers. Concentrate it under reduced pressure and then dissolve it in 5 mL of dichloromethane.

② Purification: Fill 5 g of Florisil (Heat Florisil for column chromatography overnight at 130 °C, then cool in a desiccator) and 2 g of anhydrous sodium sulfate into a glass column (internal diameter 14 mm and length 400 mm). Elute 30 mL of dichloromethane and discard the effluent. Make sure that 5 mL of the extracted solution is absorbed. Elute a 150 mL dichloromethane and ethyl acetate (49: 1) mixture and collect the effluent. Concentrate it under reduced pressure, dissolve it in 1 mL of acetonitrile, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard fenpyroximate in acetone to make 100 ppm.

② Standard solution: Dilute the standard stock solution using acetone to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 254 nm).

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 4.6 mm and length 25 cm).

Column temperature: Ordinary temperature (30 °C).

Mobile phase: A mixture of acetonitrile and water (9 : 1).

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

13) Sethoxydim

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well, and mix 10 g of it with 100 mL of diluted methanol (4 in 5). After shaking the mixture vigorously for 1 hour, filter the extracted solution under reduced pressure using a vacuum pump, a Erlenmeyer flask with branch and a Buchner funnel. After removing methanol by concentrating the extracted solution under reduced pressure, transfer it to a separatory funnel and add 50 mL of saturated sodium chloride solution and 200 mL of water. Add 70 mL of dichloromethane to the mixture, shake it vigorously, and then allow to stand to separate the layers. Transfer the bottom layer (dichloromethane layer) to another separatory funnel, add 50 mL of dichloromethane to the water layer again. After shaking the solution vigorously, allow to stand to separate the layers. Then, collect the dichloromethane layer and dry by passing an anhydrous sodium sulfate through the dichloromethane layer. Concentrate it under reduced pressure and then dissolve it in a 5 mL hexane and ethyl acetate (19:1) mixture.

② Purification: In advance, add 6 mL hexane into a silica gel cartridge [cartridge filled with 500 mg of silica gel, capacity 6 mL], allow to stand in cartridge for 2 minutes, and elute it and discard the effluent. Pour a 6 mL hexane and acetone (19:1) mixture into the cartridge and elute again in the same method. Put the extracted solution into the top of the column, allow to stand in the column for 2 minutes, and slowly elute it. While the cartridge is wet with the solvent, elute it with 5 mL of hexane-acetone mixture (9:1) and collect the effluent. Concentrate the effluent under reduced pressure on a water bath at below 40 °C, dissolve it in 2 mL of methanol, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard sethoxydim in methanol to make 100 ppm.

② Standard solution: Dilute the standard stock solution with methanol to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 280 nm).

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 4.6 mm and length 25 cm).

Mobile phase: A mixture of Methanol and water (7:1).

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

14) Fluazifop-butyl

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 10 g of it with 100 mL of acetonitrile. Homogenize it for about 3 minutes at a high speed. Filter the extracted solution under reduced pressure using a filter aid (Celite® 545) and a Buchner funnel. Transfer the filtered solution to a separatory funnel that contains 100 mL of a saturated sodium chloride solution and

400 mL of water. Add 50 mL of dichloromethane, shake the solution vigorously and allow to stand to separate the layers. After transferring the bottom layer (dichloromethane layer) to another separatory funnel, add 50 mL of dichloromethane to the water layer again. After shaking the solution vigorously, allow to stand to separate the layers.Collect the dichloromethane layer and dry by passing an anhydrous sodium sulfate through the dichloromethane layer. Concentrate it under reduced pressure and then dissolve it in a 5 mL dichloromethane, hexane and acetonitrile (1000:993:7) mixture.

② Purification: Fill 10 g of Florisil for column chromatography and 2 g of anhydrous sodium sulfate into a glass column (internal diameter 20 mm and length 30 mm) by using hexane. Rinse the column with 50 mL of hexane. After making sure that the extracted solution is absorbed into the column, elute it with a 70 mL dichloromethane, *n*-hexane and acetonitrile (100 : 97 : 3) mixture, and collect the effluent. Concentrate the effluent under reduced pressure on a water bath at below 40 °C, dissolve it in 2 mL of acetonitrile, and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard flauzifop-P-butyl in acetonitrile to make 100 ppm.

② Standard solution: Dilute the standard stock solution with acetonitrile to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Ultraviolet absorption photometer (measuring wavelength, 225 nm)

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 4.6 mm and length 25 cm).

Column temperature: Constant temperature around 40 °C.

Mobile phase: A mixture of acetonitrile and diluted formic acid (1 in 1000) (7 ： 3).

Flow rate: 1.0 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

15) Oxolinic acid

A) Preparation of the test solution

Powder 500 - 600 g of this drug well, mix 10 g of it with 30 mL of water and 5 mL of 5 mol/L hydrochloric acid, and allow to stand for 30 minutes. After adding 100 mL of acetone and homogenizing it for about 5 minutes at a high speed, filter it under reduced pressure. Transfer the filtered solution to a separatory funnel that contains 50 mL of a saturated sodium chloride solution and 450 mL of water. Add 100 mL of hexane, shake the solution vigorously, and allow it to stand to separate the layers. Discard the hexane layer and add 70 mL of dichloromethane to the water layer. Shake the solution vigorously and allow to stand to separate the layers. Transfer the bottom layer (dichloromethane layer) to another separatory funnel, and add 70 mL of dichloromethane to the water layer again. After shaking the solution vigorously, allow to stand to separate the layers. After collecting the dichloromethane layer, dry by passing anhydrous sodium sulphate through it. Concentrate it under reduced pressure, dissolve it in 10 mL of 0.01 mol/L oxalic acid-acetonitrile-methanol mixture (6:3:1), and use it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard oxolinic acid in a methanol and sodium hydroxide solution (0.25 mol/L) (9:1) to make 100 ppm.

② Standard solution: Dilute the standard stock solution with methanol to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the liquid chromatography method under the conditions below:

Operating conditions

Detector: Fluorescence photometer (excitation wavelength, 330 nm; fluorescence wavelength, 365 nm) or ultraviolet absorption photometer (measuring wavelength, 360 nm).

Column: Fill a 5 μm octadecylsilyl silica gel for liquid chromatography into a stainless steel column tube (internal diameter 4.6mm and length 25 cm).

Column temperature: Constant temperature around 40 °C.

Mobile phase: A mixture of diluted acetic acid (1 in 200), acetonitrile and methanol (7:3:1).

Flow rate: 0.8 mL/min

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

16) Pencycuron

A) Preparation of the test solution

① Extraction: Powder 500 - 600 g of this drug well and mix 20 g of it with 80 mL of acetone. After homogenizing for 5

minutes using a homogenizer, filter it under reduced pressure using a vacuum pump, an Erlenmeyer flask with branch and a Buchner funnel. Transfer the filtered solution to a 500 mL separatory funnel and add 50 mL of a saturated sodium chloride solution and 450 mL of water. Add 60 mL of dichloromethane to the mixture, shake it vigorously, and allow to stand to separate the layers. Collect the bottom layer (dichloromethane layer) and put it in another separatory funnel. After adding 60 mL of dichloromethane to the water layer and shaking vigorously, allow to stand to separate the layers, and then collect the bottom layer (dichloromethane layer). Dry by passing an anhydrous sodium sulfate through the dichloromethane layer, concentrate it under reduced pressure, and then dissolve it in 10 mL of hexane.

② Purification: Fill 10 g of Florisil [Heat Florisil (60 to 100 mesh) for column chromatography overnight at 130 ℃, then cool in a desiccator] into a glass column (internal diameter 20 mm and length 30 mm) by using hexane. Elute 50 mL of hexane and add the concentrated solution. Elute 50 mL of acetone-hexane mixture (1 in 20) and discard the effluent. Elute 60 mL of acetone-hexane mixture (1 in 20) and collect the effluent. Concentrate the effluent under reduced pressure on a water bath at below 40 °C and dissolve the residues in 1 mL of acetone.

③ Derivatization: After adding 0.5 mL of dimethyl sulfoxide to the above solution, add about 0.2 g of sodium hydride and 0.5 mL of methyl iodide. Cover it with a stopper and allow to stand for 30 minutes at 30 °C, while shaking it from time to time. Add 5 mL of hexane and shake it vigorously for about 1 minute, then add slowly 10 mL of distilled water in drops. When it stops generating hydrogen gas, transfer it to a separatory funnel using a small amount of hexane and distilled water, and shake it vigorously for 5 minutes. Dry by passing an anhydrous sodium sulfate column through the hexane layer, rinse the column with about 20 mL of hexane, and concentrate the solution under reduced pressure on a water bath at below 40 °C. Dissolve the residues in hexane, and use a certain amount of it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve standard pencycuron in acetone to make 100 ppm.

② Standard solution: Dilute the standard stock solution using acetone to an appropriate concentration

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the gas chromatography method under the conditions below:

Operating conditions

Detector: Nitrogen-Phosphorus Detector (NPD)

Column: A silicate glass capillary column tube with an internal diameter of 0.25 mm and a length of 30 m coated with 5% methylsilicon for gas chromatograph to a thickness of 0.25 μm, a silicate glass capillary column tube with an internal diameter of 0.25 mm and a length of 30 m coated with 50% phenyl and 50% methyl silicon for gas chromatograph to a thickness of 0.25 μm or equivalent

Column temperature: Constant temperature around 230°C

Sample injection port temperature: 250°C

Detector temperature: 280°C

Carrier gas: nitrogen

Flow rate: 1.0 mL/min

Split ratio: 10 ： 1

② Qualitative test

ⓐ When comparing each peak on the chromatogram obtained under the above conditions with the peak of the standard solution, the retention time for each peak must be consistent under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

17) Methidathion, Triazophos, Fenitrothion, Phenthoate

A) Preparation of the test solution

① Extraction: Powder 20 - 30 g of this drug well, mix 2 g of it with 20 mL of distilled water, and allow to stand for more than an hour. Add 100 mL of acetone to it, homogenize for 3 minutes using a homogenizer, and filter it under reduced pressure. Rinse off the residues with a small amount of acetone. After collecting the filtered solution and concentrating it under reduced pressure until 50 mL is left, transfer it to a separatory funnel. Add 500 mL of distilled water and 50 mL of a saturated sodium chloride solution and extract the solution twice using 50 mL of dichloromethane. Dehydrae by passing an anhydrous sodium sulfate through it and then concentrate the solution under reduced pressure.

② Purification: Fill 10 g of Florisil [Heat Florisil (60 to 100 mesh) for column chromatography overnight at 130°C, then cool in a desiccator] and 2 g of anhydrous sodium sulfate into a glass column (internal diameter 20 mm and length 30 mm) by using hexane. After dissolving the concentrated solution in 10 mL of hexane and transferring the solution to the column prepared in advance, elute it with 30 mL of hexane and ethyl acetate (49 : 1) mixture and discard the effluent. Elute it with 60 mL of hexane and ethyl acetate (4 : 1) mixture again and collect the effluent. Concentrate and dry the effluent under reduced pressure on a water bath at below 40 °C. Dissolve it in acetone, and use a certain amount of it as the test solution.

B) Preparation of the standard solution

① Standard stock solution: Dissolve relevant agrochemicals in acetone to make 100 ppm.

② Standard solution: Dilute the standard stock solution with acetone to an appropriate concentration.

C) Reagents and test solutions

① Water: Distilled water or equivalent

② Solvent: Solvents for testing agrochemical residues or equivalent

③ Other reagents: Reagents for testing agrochemical residues or special grade reagents

D) Procedure

① Perform the test using a test solution and a standard solution according to the gas chromatography method under the conditions below:

Operating conditions

Detector: Flame photometric detector (FPD) or Nitrogen-Phosphorus Detector (NPD)

Column: A silica glass capillary column (internal diameter 0.25 mm and length 30 m) coated with 5% phenyl and 95% methyl silicon for gas chromatography in 0.25 µm thickness, or equivalent.

Column temperature: Inject this drug at 150 °C and increase the temperature by 5 °C every minute until 260 °C. Maintain this temperature for more than 10 minutes (adjust it if necessary).

Sample injection port and detector temperature: 220 °C, 260 °C (adjust it if necessary)

Gas flow rate in detectors: Adjust the amount of hydrogen and air appropriately.

Carrier gas and flow rate: Adjust the amount of nitrogen and helium appropriately.

② Qualitative test

ⓐ The peak in the chromatogram obtained under the above conditions must be consistent with the peak obtained in the standard solution under any measurement condition.

ⓑ Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

③ Quantitative test: It is done according to the peak height or peak area measurement method, based on the results obtained under the same conditions as the qualitative test.

18) Assessment

A) Follow the specifications stated in the monograph. Unless otherwise specified, the limit of agrochemicals in crude drugs and crude drug extracts is as follows: Total amount of DDT (including p,p'-DDD, p,p'-DDE, o,p'-DDT and p,p'-DDT) must be below 0.1 ppm, dieldrin must be below 0.01 ppm, the total amount of BHC (including a, ß, γ and d-BHC) must be below 0.2 ppm, aldrin must be below 0.01 ppm and endrin must be below 0.01 ppm (inspection can be exempted for the crude drug extracts whose raw material has been tested).

B) If agrochemicals that are not listed in Clause A) have been detected, the following items are applicable for a suitability assessment.

① The suitability assessment should be done in accordance with Clause "PESTICIDE RESIDUES" of the European Pharmacopoeia.

② When agrochemicals that are not listed in the European Pharmacopoeia are detected, the Minister of the Ministry of Food and Drug Safety can assess the suitability using the following formula:

$$\frac{ADI \times M}{MDD \times 100}$$

ADI: Permitted daily intake of the relevant agrochemicals (mg/kg/day)

M: Average adult weight (60 kg)

MDD: Daily dosage of the relevant crude drug (kg)

C) Despite Clause B), in the following cases, suitability is determined according to the criteria specified in each subparagraph.

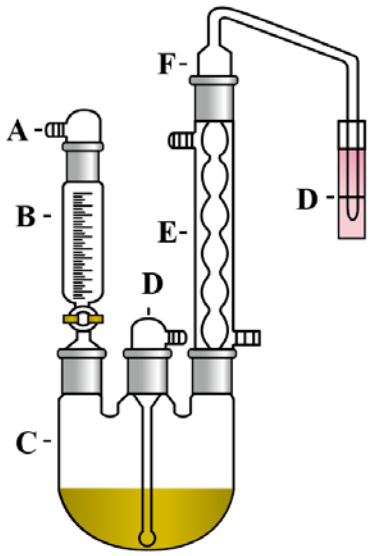
① If a crude drug item contains agrochemical residues other than those that comply with the guidelines for permitted residue levels of agrochemicals (announced by the Ministry of Food and Drug Safety), then follow the "Standards for Agrochemical Residues in Agricultural Products" and the "Provisional Standards for Agrochemical Residues in Processed Food" in the "Standards and Specifications of Foods" (announced by the Ministry of Food and Drug Safety).

② If "Citrus Unshiu Peel" and "Immature Citrus Unshiu Peel" are found to contain agrochemical residues that are listed as permitted agrochemical residues for tangerines in "Standards and Specifications of Foods" (announced by the Ministry of Food and Drug Safety), the limit will be determined by multiplying the level of "tangerines" by each processing factor (Citrus Unshiu Peel, 8; Immature Citrus Unshiu Peel, 14)

D. Sulfur dioxide

1) Apparatus

Use the apparatus shown in the Figure.



Figure

A: Hose connector
B: Separatory funnel (capacity of 100 mL or more)
C: Distilling flask (1000 mL) D: Gas injection tube
E: Allihn condenser (300 mm) F: Bubbler
G: Gas collector (internal diameter 25 mm and depth 150 mm)

2) Test solution

A) *Methyl red test solution*: Dissolve 250 mg of methyl red in ethanol to make 100 mL.

B) 3% Hydrogen peroxide solution: Add water to 10 mL of hydrogen peroxide (30) to make 100 mL, add 3 drops of methyl red test solution, and add 0.01 mol/L sodium hydroxide solution to make a light yellow solution (prepare it before use).

3) Procedure

Add 400 mL of water to the distilling flask (C). Lock a cork in the separatory funnel (B) and add 90 mL of 4 mol/L hydrochloric acid. Pass nitrogen gas through the gas injection tube (D) at a speed of 0.21 L/min, and pass cold water through the Allihn condenser (E). Add 30 mL of 3% hydrogen peroxide solution to the collector (G). Remove the separatory funnel (B) after 15 minutes. Mix 50 g of sample powder and 100 mL of water and ethanol mixture (95 : 5), and put the mixture into the distilling flask (C). After reconnecting the separatory funnel (B), open the

cork and pour 4 mol/L hydrochloric acid into the distilling flask (C), and leave 2 - 3 mL in the separatory funnel. Lock the cork and heat the distilling flask. After 1 hour and 45 minutes from boiling the mixture in the distilling flask, remove the collector (G) and rinse the end of the bubbler (F) with a small amount of hydrogen peroxide solution. Pour this into the collector (G) and carry out titration with 0.01 mol/L sodium hydroxide solution using a micro buret. Titrate until the solution turns yellow and remains yellow for more than 20 seconds (V1 mL). Perform a blank test in the same way (V_2 mL).

Each mL of 0.01 mol/L sodium hydroxide solution
= 320 μg of SO_2

$$Sulfur\ dioxide(mg/kg) = \frac{320 \times (V_1 - V_2) \times f}{S}$$

V_1: Consumption of 0.01 mol/L sodium hydroxide solution (mL)

V_2: Consumption of 0.01 mol/L sodium hydroxide solution during a blank test (mL)

f: Potency of 0.01 mol/L sodium hydroxide solution

S: Amount of sample taken (g)

E. Mycotoxin

1) Preparation of the test solution

Powder 500 - 600 g of this drug well, weigh 5.0 g accurately, and mix it with 100 mL of diluted methanol (7 in 10). After extracting by sonication for 30 minutes, filter it. Add diluted methanol (7 in 10) accurately to make a 100 mL solution, take exactly 10 mL of this solution again, dilute it with water to make 80 mL, and use it as the extracted solution.

Pass 40 mL of the extracted solution through an immunoaffinity column (for aflatoxin) and pass 10 mL of water through the column twice at a speed of 3 mL/min. Discard the effluent. Dry by passing a weak vacuum through immunoaffinity column (for aflatoxin) for 5 - 10 seconds or by passing air through a syringe for 10 seconds. Add 0.5 mL of methanol to the dried immunoaffinity column (for aflatoxin) and allow the effluent to come out by gravity. Allow to stand for 1 minute and then pass 0.5 mL of methanol twice. Collect all the effluent and add water to make it 5 mL. Make sure that the flow rate does not exceed 5 mL/min. If the effluent is clear, use it as the test solution, and if necessary, filter it with a 0.45 μm filter.

2) Preparation of the standard solution

Accurately weigh about 1.0 mg of each of the standard aflatoxin B1, B2, G1 and G2, respectively, mix it with a toluene and acetonitrile (98 : 2) mixture to make 100 mL, and use it as the first standard stock solution of the aflatoxin mixture. Mix 1 mL of this first standard stock solution with the toluene and acetonitrile (98 : 2) mixture to make 100 mL, and use it as the second standard stock solution.

3) Calibration curve drawing

Prepare a standard solution by diluting the above standard stock solution to an appropriate concentration and draw a calibration curve. If the concentration of the test solution is outside the range of the calibration curve, adjust the concentration of the standard solution make it within the range.

4) Procedure

Use 10 - 500 μL of the test solution and standard solution to test according to the liquid chromatography method. Measure the peak areas of each aflatoxin in the test solution. Calculate the amount of each aflatoxin in the test solution using the calibration curve made from the peak areas, and use the following formula to calculate the total amount of aflatoxin (B1, B2, G1, G2).

The total amount of aflatoxin (B1, B2, G1, G2) in the sample
$$= \frac{V_1 \times V_2 \times (C_{B1} + C_{B2} + C_{G1} + C_{G2})}{\mathrm{m} \times V_i}$$

m = Amount of sample collected in dry state (g)

V_1 = Amount of solvent used during extraction (mL)

V_i = Effluent used for immunoaffinity column for aflatoxin (mL)

V_2 = Final volume of the solution diluted with water after elution from the immunoaffinity column for aflatoxin (mL)

C_{B1} = Aflatoxin B1 concentration measured in the test solution (ng/mL)

C_{B2} = Aflatoxin B2 concentration measured in the test solution (ng/mL)

C_{G1} = Aflatoxin G1 concentration (ng/mL) measured in the test solution

C_{G2} = Aflatoxin G2 concentration measured in the test solution (ng/mL)

Operating conditions

Detector: Fluorescence photometer (excitation wavelength, 365 nm; fluorescence wavelength, 435 nm)

Column: A stainless steel column tube (internal diameter 4.6 mm and length 15 - 25 cm) coated with octadecylsilyl silica gel for liquid chromatography in 3 - 5 μm thickness, or equivalent.

Mobile phase A: A mixture of water, methanol and acetonitrile (6:3:2)

Mobile phase B: Solution containing 0.12 g of potassium bromide per liter and 350 μL of dilute nitric acid per liter of mobile phase A.

Flow rate: 1.0 mL/min

Post-column derivatization apparatus: Any of the following three can be selected for the test.

A) Pyridium hydrobromic acid perbromate (PBPB)

① Pulseless Pump

② Polytetrafluoroethylene reaction tube: 0.45 m × 0.5 mm

③ Mobile phase: Mobile phase A

④ Derivatization solution: Dissolve 50 mg of pyridium hydrobromide perbromate in 1000 mL of water, store it away from light and use it within 4 days.

⑤ Flow rate of the derivatization solution: 0.4 mL/min

B) Photochemical Reactor for Enhanced Detection (PHRED)

① 254 nm low pressure mercury UV lamp (minimum 8 W)

② Polished support plate

③ Knitted reactor coil: Polytetrafluoroethylene tubing around the UV lamp

④ Exposure time: 2 min

⑤ Mobile phase: Mobile phase A

C) Electrochemically generated bromine (KOBRA)

① KOBRA cell: An electrochemical cell to generate

an activated form of bromine by derivatizing aflatoxin and amplifying its fluorescence

② DC induction device connected to KOBRA cell: power supply of about 100 uA

③ Polytetrafluoroethylene reaction tube: 0.12 m × 0.25 mm

④ Mobile phase: Mobile phase B

Note) After purchasing and storing a standard product for a certain period of time, the concentration of the standard product must be adjusted by carrying out the following procedures before use.

Accurately weigh about 1.0 mg of standard aflatoxin B1 and add a mixture of toluene and acetonitrile (98 : 2) to make a 100 mL solution, and use it as the first standard stock solution of aflatoxin B1. With this standard stock solution, measure optical density (between 330 - 370 nm) according to the Ultraviolet-visible Spectroscopy listed in the general test of the Korean Pharmacopoeia, and calculate the concentration (μg/mL) of the first standard stock solution of aflatoxin B1 using the following formula:

$$\text{Concentration (μg/mL) of the first standard stock solution of aflatoxin B1 } (C_{17}H_{12}O_6) = \frac{A \times M \times 100}{\epsilon \times \zeta}$$

A = Absorbance at the maximum of the absorption curve

M = Molecular weight of aflatoxin B1: 312 g/mol

ε = Molar absorptivity of aflatoxin B1 in the toluene and acetonitrile mixture: 1930 m2/mol

ζ = Optical range of the cell used to measure optical density: 1 cm

Note) Store the standard stock solution away from light and at below 4 °C. Do not remove the aluminum foil until the temperature of the solution reaches the ordinary temperature.

F. Benzopyrene

Powder or chop finely 500 - 600 g of this drug, mix homogeneously, accurately weigh about 5.0 g accurately, and mix it with 100 mL of water. After extracting the solution for 90 minutes using ultrasonic waves, add 100 mL of hexane and 1 mL of the internal standard solution. Blend it with a homogenizer for 5 minutes and extract it for 30 minutes using ultrasonic waves. After transferring the hexane layer to a separatory funnel, add 50 mL of hexane to the water layer. Extract it by shaking, and repeat this process again. Collect the hexane layer after the second process and add it to the separatory funnel. Add 50 mL of water to the hexane layer and combine in the separatory funnel. After drying and filtering the hexane layer using filter paper that contains anhydrous sodium sulphate, concentrate hexane at lower pressure (about 700 mbar) in water bath at 45 °C until about 2 mL of hexane is left. Use a Florisil cartridge that has been activated in advance by eluting 10 mL of dichloromethane and 20 mL of hexane in order at a speed of 2 - 3 drops per second. Add the extracted solution into the previously activated cartridge and effuse a 20 mL hexane and dichloromethane (3: 1) mixture at a speed of 2 - 3 drops per second. After drying the effluent with nitrogen gas on a water bath at below 35 °C and dissolving the residues in 1 mL of acetonitrile, filter it with a membrane filter (pore size of 0.45 μm or less), and use it as the test solution. Accurately weigh an appropriate amount of standard benzopyrene and standard 3-methylcholanthrene, and dissolve them in acetonitrile, separately, to make 1 μg/mL of the standard stock solution and the internal standard stock solution. Store the standard stock solution and the internal standard stock solution at 5 - 15 °C and use them within 30 days. Take accurately an appropriate amount of the standard stock solution and the internal standard stock solution, and dilute them with acetonitrile to contain 3, 5, 10, 20, and 40 ng of benzopyrene as well as 50 ng of internal standard substance per mL respectively, to make the standard solution. If the concentration of the test solution is outside the range of the calibration curve, adjust the concentration of the standard solution to be within the range of the calibration curve. Perform the test with 10 μL of the test solution and standard solution according to the liquid chromatography method under the following conditions. Create a calibration curve by setting the ratio of the peak area of benzopyrene to the peak areas of the internal standard matter [AS/AIS], obtained from each standard solution, as the Y-axis, and setting the concentration of benzopyrene as the X-axis. Find the concentration of benzopyrene by plugging the peak area ratio [ASAM/ASAMIS] of benzopyrene to the peak areas of the internal standard substance, obtained from the test solution, on the Y-axis.

A_S: Standard substance peak area of the calibration curve standard solution

A_{IS}: Internal standard substance peak area of the calibration curve standard solution

A_{SAM}: Benzopyrene peak area of the test solution

A_{SAMIS}: Peak area of the internal standard substance of the test solution

Internal standard solution: Weigh accurately 3-methylcholantrene reference standard, and dissolve it in acetonitrile to make a 50 ng/mL solution.

Reagents and test solutions: Water used IN this test must be a tertiary distilled water or more, and reagents must be agrochemical residues used for testing or more.

Operating conditions

Detector: Fluorescence photometer (excitation wavelength, 294 nm; fluorescence wavelength, 404 nm)

Column: Supelcosil LC-PAH (4.6 × 250 mm, 5 μm) or equivalent

Column temperature: 37°C

Mobile phase: A mixture of acetonitrile and water (8:2)

Flow rate: 1.0 mL/min

Loss on drying

Unless otherwise specified, accurately weigh 2 – 6 g of the test specimen using a weighing bottle (weighed in advance). Dry at 105 °C for 5 hours and cool in a desiccator (silica gel), then weigh it again accurately. Dry it again at 105 °C and weigh it every hour until the weight remains constant. Reduction in weight is the loss on drying (%). If the drying time is specified, loss on drying (%) can be calculated by the reduction in weight during the set period of time.

Total ash

Ignite a platinum, quartz or ceramic crucible at 500 - 550 °C for 1 hour, leave it to cool down, and then weigh it accurately. Unless otherwise specified, place 2 - 4 g of the test specimen in the crucible and weigh it precisely. If necessary, cover the crucible fully or partially with a lid. Start to heat the crucible at a

low temperature and gradually increase the temperature. Incinerate it by heating for 4 hours or more at 500 - 550 °C until no carbide is left. Leave it to cool down and weigh the ash precisely. Incinerate it again and repeat this process until the weight remains constant. Weigh the ash precisely to determine the ash content (%). If carbide remains and a constant weight cannot be achieved using this method, add boiling water and extract. Filter it using a quantitative filter paper, and ignite the residue, filter paper, and the insoluble matter on the filter paper until no carbide is left. Add the filtrate to this and dry it through evaporation. Ignite, let it cool down, and then weigh it precisely to determine the ash content (%). If carbide remains, wet it with a small amount of ethanol and break down the carbide with a glass rod. Then rinse the glass rod with a small amount of ethanol before carefully evaporating the ethanol and weighing the residue using the same method above. Let it cool down in a desiccator (silica gel).

Acid-insoluble ash

Add 25 mL of diluted hydrochloric acid to ash carefully and boil it at a low heat for 5 minutes. Filter the insoluble matter using a quantitative filter paper and rinse the residue with boiling water. Dry it with the filter paper and ignite for 3 hours using the same method as described in the total ash section. Let it cool down in a desiccator (silica gel) and weigh it precisely to calculate the acid-insoluble ash content (%). If the calculated value is larger than the specified value, ignite it again until a constant weight is reached.

Extract content

Perform the test according to the following method.

1) Dilute ethanol-soluble extraction

Unless otherwise specified, accurately weigh about 2.3 g a of the test specimen, place it into an appropriate flask and add 70 mL of diluted ethanol. Extract it for 5 hours, and shake it from time to time. Allow to stand for 16 - 20 hours and filter it. Rinse off the residue from the flask with diluted ethanol until the filtrate reaches100 mL. Evaporate 50 mL of the filtrate to dryness in water bath, dry it at 105 °C for 4 hours, and cool it down in a desiccator (silica gel). weigh it precisely and calculate the amount of the diluted ethanol extraction by multiplying the weight by 2. From the value obtained from the loss on drying, calculate the extraction amount (%) converted into the amount of the dry specimen.

2) Water-soluble extraction

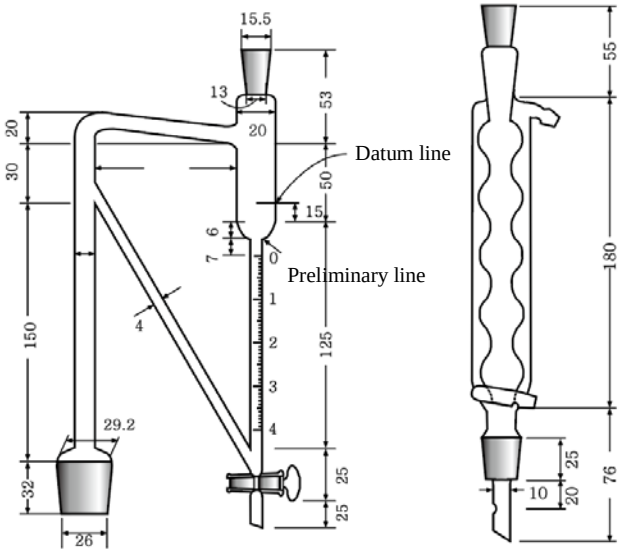
Follow the same method used in 1) Diluted ethanol, and use water instead of diluted ethanol to measure the weight precisely. Multiply the weight by 2 to obtain the amount of water extraction. From the value obtained from the loss on drying, calculate the extraction amount (%) converted into the amount of the dry specimen.

3) Ether Extract

Unless otherwise specified, dry the test specimen in a desiccator (silica gel) for 48 hours. Accurately weigh about 2 g and place it into an appropriate flask, add 70 mL of ether into the flask, and connect with reflux condenser. Boil it at a low temperature in water bath for 4 hours before cooling it down, and filter it. Rinse the residue and the flask with ether until the filtrate reaches 100 mL. Evaporate 50 mL of the filtrate to dryness on a water bath and dry it in a desiccator (silica gel) for 24 hours. Weigh it accurately and multiply it by 2 to find the amount of the ether extraction. Calculate the extraction amount (%).

Essential oil content

Put the amount of the test specimen, specified in the monograph, into a hard-glass flask with a capacity of 1000 mL. Add 5 to 10 times the amount of water and install an essential oil volume measure (Fig.1). Connect a reflux condenser to the top end of the measure (Fig.2) and carefully boil it in an oil-bath at 130 - 150 °C. Fill the graduated tube of the measure with water to the datum line and add 2.0 mL of xylene. Unless otherwise specified, boil it for 5 hours and allow to stand for a short time. After opening the cork of the measure, remove water slowly until the top end of the oil layer reaches the preliminary line of the graduated tube. Allow to stand for 1 hour at ordinary temperature and lower the top of the oil layer to the zero line of the graduated tube. Measure the amount of the oil layer (mL) at ordinary temperature and subtract the amount of xylene to calculate the amount of the essential oil in the crude drug.



*The figures are in mm.

Figure 1 **Figure 2**

Crystallinity
결정성시험법

The Crystallinity is to measure the crystallinity of the sample using a polarizing microscope.

Procedure

Float the sample on a mineral oil and perform the test using a suitable polarizing microscope. Add 1 to 2 drops of liquid paraffin to a small amount of the sample, shake lightly to mix, and then observe by rotating the polarizing microscope 90° each time. If the sample is a fine powder, use the oil immersion technique to examine it. If the sample has crystallinity, birefringence and quenching are observed.

Digestive Power
소화력시험법

The Digestive Power is to measure the digestion activity of

drug substances or preparations containing digestive enzymes (starch digestion activity, protein digestion activity, fat digestion activity).

Assay for starch digestion activity

The measurement of starch digestion activity is performed according to starch saccharifying activity, dextrinizing activity, and liquefying activity tests.

1) Measurement of starch saccharifying activity

starch saccharifying activity can be determined by measuring the increase in reducing activity due to the hydrolysis of glucoside linkages when amylase acts on the starch. When tested under the operating conditions, one starch saccharifying activity unit is the amount of enzyme required to increase the reducing activity, which is equivalent to 1 mg of glucose per minute.

Preparation of the test solution

Weigh an appropriate amount of the sample, dissolve it in an appropriate amount of water, or a buffer or a salt solution specified in the monograph to obtain a concentration in a range proportional to the increase in the reducing activity and use it as the test solution. The concentration is usually 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

Preparation of the substrate solution

Use potato starch TS to measure the starch digestion activity. If necessary, add 10 mL of the buffer or salt solution specified in the monograph instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0).

Procedure

Take exactly 10 mL of the substrate solution, heat it at 37 ± 0.5 °C for 10 minutes, add exactly 1 mL of the test solution, and shake to mix. Allow to stand at 37 ± 0.5℃ for exactly 10 minutes, then add exactly 2 mL of Fehling's alkaline tartrate TS for the starch digestion assay, and shake to mix. Then, add exactly 2 mL of Fehling's copper TS for the starch digestion assay, shake gently, heat the solution on a water bath for exactly 15 minutes, and cool immediately to below 25℃ with running water. Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of dilute sulfuric acid (1 in 6), and titrate the released iodine with 0.05 mol/L sodium thiosulfate solution (a mL). The endpoint is set as the time of the disappearance of blue color, which is created by adding 1 to 2 drops of soluble starch TS around the endpoint. Separately, titrate with 10 mL of water instead of the substrate solution and titrate in the same way (b mL).

$$\text{Starch saccharifying activity (unit/g)} = \text{amount(mg)of glucose} \times \frac{1}{10} \times \frac{1}{M}$$

$$\text{Amount (mg) of glucose} = (b - a) \times 1.6$$

M: Amount (g) of sample in 1 mL of the test solution

2) Measurement of starch dextrinizing activity

The starch dextrinizing activity can be determined by measuring the decrease in starch coloration caused by iodine due to the hydrolysis of the straight chain component (amylose) in starch when amylase acts on the starch. When testing under the operating conditions, one dextrinizing activity unit is the amount of enzyme required to decrease the coloration of potato starch by iodine by 10% per minute.

Preparation of the test solution

Weigh an appropriate amount of the sample, dissolve it in an appropriate amount of water or a buffer or a salt solution specified in the monograph so that the coloration of the starch caused by iodine decreases in proportion to the concentration of the test solution, when measured under the operating conditions. The concentration is usually 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

Preparation of the substrate solution

Perform the same procedures as the substrate solution when measuring the starch saccharifying activity.

Procedure

Take 10 mL of the substrate solution, heat at 37 ± 0.5℃ for 10 minutes, add exactly 1 mL of the test solution, and shake immediately to mix. Allow to stand at 37 ± 0.5℃ for exactly 10 minutes. Then, accurately take 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately to mix. Take 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS and shake. Determine the absorbance A_T of this solution at a wavelength of 660 nm under the Ultraviolet-visible Spectroscopy, using water as the blank. Separately, determine the absorbance A_B in the same manner using 1 mL of water instead of the test solution.

$$\text{Dextrinizing activity (unit/g)} = \frac{A_B - A_T}{A_B} \times \frac{1}{M}$$

M: Amount (g) of sample in 1 mL of the test solution

3) Measurement of starch liquefying activity

The starch liquefying activity can be determined by measuring the decrease in viscosity of the starch solution due to the hydrolysis of molecules when amylase acts on the starch. When testing under the operating conditions, one starch liquefying activity unit is the amount of the enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch in 1 minute from 2 times the viscosity of 50% sucrose standard solution to 1 time.

Preparation of the test solution

Weigh an appropriate amount of the sample, dissolve it in an appropriate amount of water or a buffer or a salt solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the test solution when measuring under the operating conditions, and use it as test solution. The concentration of the test solution is usually 0.15 to 0.25 starch liquefying activity unit/mL.

Preparation of the substrate solution

Accurately weigh about 1 g of potato starch, heat at 105℃ for 2 hours, and measure the loss on drying. Accurately weigh 15.00 g of potato starch on a dried basis, add 300 mL of water, then gradually add 25 mL of 2 mol/L sodium hydroxide TS, and shake until the mixture forms a paste. Heat the mixture on a water bath for 10 minutes, while shaking occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

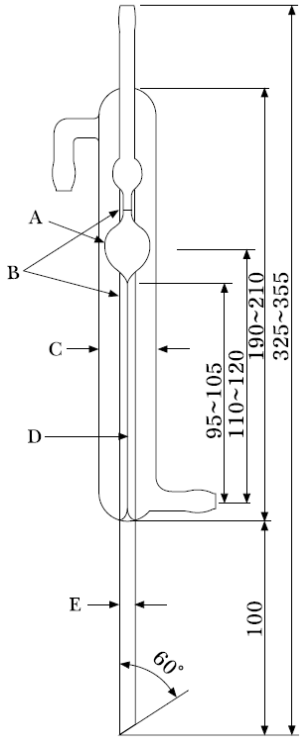
Preparation of the 50% standard sucrose solution

Dissolve 50.0 g of sucrose in 50 mL of water.

Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL Erlenmeyer flask and allow to stand on a water bath at 37 ± 0.5℃ for 15 minutes. Attach the viscometer shown in Figure 1 vertically for its lower end to touch nearly the bottom of the flask, and let the water of the water bath circulates around the outer cylinder of the viscometer. Slowly draw the 50% standard sucrose solution by suction into the center of the upper bulb of the viscometer and allow it to flow downward by gravity. Measure the time (t_1) it takes for the solution to flow down from the upper

to the lower gauge line. Add exactly 50 g of the substrate solution to another 100 mL Erlenmeyer flask and transfer it to another water bath at 37 ± 0.5℃ for 20 minutes. Add exactly 1 mL of the test solution and shake the flask immediately. Attach the viscometer vertically for its lower end to touch nearly the bottom of flask, and let the water of the water bath flow into the cylinder out of the viscometer. Occasionally, draw the reaction solution up slowly by sucking it to the center of the upper bulb, then let it flow down by gravity and measure the time (*t*) it takes for the solution to flow down from the upper to lower gauge line. Repeat this process until *t* is shorter than t_1. For each measurement, record the time (*T*') between the addition of the test solution and the moment when the surface of the solution in the flask passes the upper gauge line. ($T' + t/2$) is the reaction time (*T*) corresponding to *t*. Draw a curve for both *t* and *T*. Find T_1 and T_2, which correspond to t_1 and ($2 \times t_1$), by interpolation.



* The figures are in mm.

Figure 1. Viscometer for determination of liquefying power

A: Bulb volume 5 mL B: Gauge line
C: Outer diameter 30 mm
D: Capillary tube internal diameter 1.25 - 1.30 mm
E: Outer diameter 8 mm

$$\text{Liquefying power (unit/g)} = \frac{60}{T_1 - T_2} \times \frac{1.5}{M}$$

M: Amount (g) of sample in 1 mL of the test solution

Assay for protein digestion activity

Protein digestion can be determined colorimetrically using Folin's reaction, and it is measured from the amount of acid-soluble low-molecular-weight products that increase due to the cleavage of peptide bonds when protease acts on casein. One protein digestion unit is the amount of enzyme that represents an increase in the Folin's TS-stainable substance equivalent to 1 μg of tyrosine per minute, under the operating conditions.

Preparation of the test solution

Under the operating conditions, weigh an appropriate amount of the sample, dissolve it in an appropriate amount of water or a buffer or a salt solution specified in the monograph to obtain a concentration in a range proportional to the increase in the non-proteinaceous Folin's TS-stainable substance, and use it as the test solution. The concentration of the test solution is usually 15 to 25 protein digestion activity unit/mL.

Tyrosine calibration curve

Weigh exactly 50 mg of the tyrosine reference standard, dry it at 105 °C for 3 hours, and dissolve it in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Take 1 mL, 2 mL, 3 mL, and 4 mL of this solution accurately, and add 0.2 mol/L hydrochloric acid TS to each to make exactly 100 mL. Take 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each, shake immediately, then let them stand at 37 ± 0.5℃ for 30 minutes. Determine the absorbance, A_1, A_2, A_3, and A_4, of these solutions at 660 nm as described under the Ultraviolet-visible Spectrophotometry, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same way as the blank. Then, draw a calibration curve by putting the absorbance A_1, A_2, A_3, and A_4 at the vertical axis and the amount (μg) of tyrosine in 2 mL of each solution at the horizontal axis. Calculate the amount (μg) of tyrosine when the absorbance difference is 1.

Preparation of the substrate solution

Substrate solution 1

Weigh exactly 1 g of milk casein, dry at 105℃ for 2 hours, and measure the loss on drying . Weigh exactly an amount of milk casein equivalent to 1.20 g, calculated on the dried basis, add 12 mL of lactic acid TS and 150 mL of water, and warm on a water bath to dissolve. After cooling with running water, adjust the pH to the value specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Substrate solution 2

Weigh accurately about 1 g of milk casein, dry at 105℃ for 2 hours, and measure the loss on drying. Weigh exactly an amount of milk casein equivalent to 1.20 g, calculated on the dried basis, add 160 mL of 0.05 mol/L disodium hydrogen phosphate TS, and warm on a water bath to dissolve. After cooling with running water, adjust the pH to the value specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Preparation of the precipitation reagent

Trichloroacetic acid TS A

Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.

Trichloroacetic acid TS B

Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5 mL of 6 mol/L acetic acid TS in water to make 100 mL.

Procedure

Take exactly 5 mL of the substrate solution specified in the monograph, warm on a thermostatically controlled water bath at 37 ± 0.5℃ for 10 minutes, add exactly 1 mL of the test solution, and shake immediately to mix. Allow this solution to stand at 37 ± 0.5 °C for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, and shake to mix. Again, allow to stand at 37 ± 0.5 °C for 30 minutes, and then

filter it. Discard the first 3 mL of the filtrate, and collect the following 2 mL of the filtrate accurately. Add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to the solution, shake well, and allow it to stand at 37 ± 0.5℃ for 30 minutes. Determine the absorbance A_T of this solution at 660 nm under the Ultraviolet-visible Spectrophotometry, using water as the blank. Separately, take 1 mL of the test solution accurately, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake to mix. To this solution, add exactly 5 mL of the substrate solution as specified in the monograph, shake immediately, and allow to stand at 37 ± 0.5℃ for 30 minutes. Perform the same procedures as the test solution and determine the absorbance A_B at 660 nm.

$$\text{Protein digestion activity (unit/g)} = (A_T - A_B) \times F \times \frac{11}{2} \times \frac{1}{10} \times \frac{1}{M}$$

M: Amount (g) of sample in 1 mL of the test solution

F: Amount (μg) of tyrosine for Absorbance difference of 1 determined from Tyrosine Calibration Curve

Assay for Fat Digestion

Fat digestion can be determined by measuring the amount of fatty acid formed during cleavage of the ester bond when lipase acts on olive oil. When tested according to the operating conditions, one fat digestion unit is the amount of enzyme that represents an increase of 1 μmol of fatty acid per minute.

Preparation of the test solution

When testing according to the operating conditions, weigh an appropriate amount of the sample, dissolve or suspend it in an appropriate amount of cold water (less than 10℃) or a buffer or salt solution specified in the monograph to obtain a concentration in a range proportional to the increase in the amount of fatty acid,and use it as the test solution. The concentration of the test solution is usually 1 to 5 fat digestion activity unit/mL.

Preparation of the substrate solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender, as shown in Figure. 2, and emulsify it for 10 minutes at 12,000 to 16,000 rpm while cooling the solution to a temperature below 10℃. Let this solution stand in a cold place for 1 hour, and make sure that the oil layer is not separated.

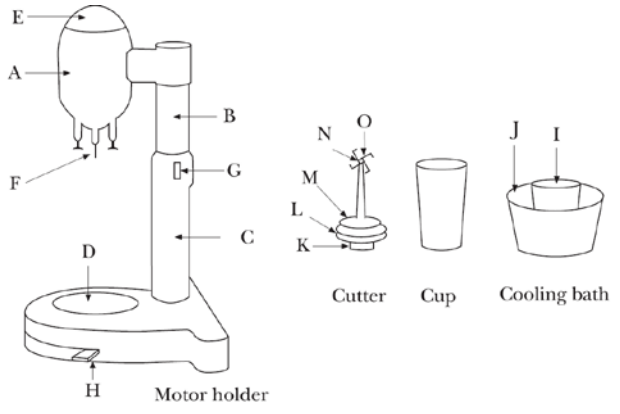


Figure 2. Blender for fat digestion

A: Motor case
B: Inner column
C: Outer column
D: Cooling bath mounting base
E: Motor head
F: Motor shaft
G: Motor ascend/descend lever
H: Rotation controller
I: Cup mounting base
J: Cooling bath
K, M: Auxiliary lid
L: Cup lid
N: Blade
O: Screw

Preparation of the emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between 75℃ and 80℃ for 1 hour while stirring. After cooling, filter the solution if necessary and add water to make exactly 1000 mL.

Procedure Take 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph into an Erlenmeyer flask and shake. Allow to stand at 37 ± 0.5 °C for 10 minutes, add exactly 1 mL of the test solution and shake immediately to mix. Let this solution stand at 37 ± 0.5 °C for exactly 20 minutes, add 10 mL of a mixture of ethanol and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide solution, add 10 mL of a mixture of ethanol and acetone (1:1), and shake. Titrate the excess sodium hydroxide with 0.05 mol/L hydrochloric acid solution (*b* mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, take accurately 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph into an Erlenmeyer flask and shake to mix. Allow to stand at 37 ± 0.5 °C for 10 minutes, add 10 mL of a mixture of ethanol and acetone (1:1), then add exactly 1 mL of the test solution and shake to mix. Add exactly 10 mL of 0.05 mol/L sodium hydroxide solution and titrate in the same way (*a* mL).

$$\text{Fat digestion activity (unit/g)} = 50 \times (a - b) \times \frac{1}{20} \times \frac{1}{M}$$

M: Amount (g) of sample in 1 mL of the test solution

Dimethylamiline
디메틸아닐린시험법

The Dimethylamiline is a limit test method for N,N-dimethylaniline present in pharmaceuticals. In the pharmaceutical monograph, the limit of dimethylaniline is expressed in ppm ( ).

Examine using gas chromatography (2.2.28), with N,N-diethylaniline as the internal standard.

Procedure

Dissolve 0.50 g of the substance to be examined in 30.0 mL of water in a ground-glass-stoppered tube. Add 1.0 mL of the internal standard solution. Adjust the solution to a temperature of 26-28 °C. Add 1.0 mL of strong sodium hydroxide solution and mix until completely dissolved. Add 2.0 mL of trimethylpentane. Shake for 2 min and allow the phases to separate. Use the upper layer as the test solution. Separately, dissolve 50.0mg of N,N-dimethylaniline accurately in 4.0 mL of 0.1 M hydrochloric acid, and dilute to 50.0 mL with water. Dilute 1.0 mL of this solution to 100.0 mL with water. Further, dilute 1.0 mL of this solution to 30.0 mL with water. Add 1.0 mL of the internal standard solution,1.0 mL of strong sodium hydroxide solution, and 2.0 mL of trimethylpentane. Shake for 2 min, allow the phases to separate, and use the upper layer as the reference solution.

$$\text{Amount (ppm) of dimethylaniline} = \text{Amount of N,N-dimethylaniline (mg)} \times (Q_T / Q_S) \times (\text{Purity of N,N-dimethylaniline (\%)} / W_T) \times 4$$

W_T: Amount (mg) of the substance

Internal standard solution: Dissolve 50mg of N,N-diethylaniline in 4 mL of 0.1 M hydrochloric acid and dilute to 50 mL with water. Take 1 mL of this solution and dilute it to 100 mL with water.

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A fused silica column with an internal diameter of 0.32 mm and a length of 30 m, coated with 50% phenyl-50% methylpolysiloxane with a thickness of 0.52 ㎛.

Column temperature: Maintain the temperature at 150℃ for 5 minute after injection, then increase to 275℃ at a rate of 20℃ per minute, finally, maintain at 275℃ for 3 minutes.

Injection port: Use a split-liner consisting of a column about 1 cm long packed with diatomaceous earth for gas chromatography impregnated with 10% m/m of methylpolysiloxane, and maintain a constant temperature of about 220℃.

Detector temperature: Keep a constant temperature of about 300℃.

Carrier gas: Helium.

Flow rate: 1.0 mL/minute (50 kPa)

Split ratio: 1:20

System suitability

System performance: When operating with 1 μL of the standard solution under the specified conditions; *N*,*N*-dimethyaniline the internal standard are eluted in this order a resolution being NLT 4.0.

System repeatability: Conduct the test 6 times with 1 μL of the standard solution each time under the specified conditions. The relative standard deviation of the ratios of the peak area of *N*,*N*-dimethylaniline to that of the internal standard should not exceed 3.0%.

Disintegration
붕해시험법

The Disintegration is performed to determine whether tablets, capsules, granules, suppositories or pills disintegrate under the designated condition within the prescribed time when placed in the test solution. The purpose of the Disintegration is not to identify whether the active ingredient in the formulation solubilizes completely. Complete disintegration means that the sample is in a soft state with no hard lumps remaining on the mesh of the glass column or attached to the lower surface of the auxiliary plate (excluding fragments of the insoluble coating or capsule shells).

Apparatus

The apparatus consists of a flat bottom beaker with a height of 138 to 160 mm, internal diameter of the immersion part of 97 to 115 mm and volume of 1000 mL, a thermostat which is adjustable to 37 ± 2 ℃ and a test apparatus and motor which move back and forth 29 to 32 times per minute and up and down with an amplitude of 53 to 57 mm. The volume of the test solution in the beaker should be such that when the test apparatus is at the highest point of the upward stroke, the wire mesh remains at least 15 mm below the surface of the fluid and descends to NLT 25 mm from the bottom of the beaker on the downward stroke. The top of the test apparatus should not be completely submerged. The time required for the motor to move upward should be equal to the time required for it to move downward. The change in direction should be smooth, avoiding abrupt reversals of motion. The test apparatus moves vertically along its axis. It should not move horizontally.

Test apparatus

The test apparatus (basket-rack assembly) consists of 6 open-ended transparent glass columns, each 77.5± 2.5 mm long and having an internal diameter of 20.7 to 23.0 mm and a wall thickness of 1.0 to 2.8 mm, and 2 plastic plates to hold such tubes in a vertical position, each 88 to 92 mm in diameter and 5 to 8.5 mm in thickness. On these plates, there are 6 holes, each 22 to 26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. On the bottom surface of the lower plastic plate, there is a flat stainless steel mesh, in which the sieve gap is 1.8 - 2.2 mm and the wire diameter is 0.57 - 0.66 mm. The parts of the test apparatus are assembled and rigidly held by 3 props running through the 2 plastic plates. The test apparatus is appropriate for the structure in Figure 1. The glass column and mesh should conform to the specifications, but other parts can be altered to a certain extent. For example, to secure the glass columns to the apparatus, an acid-resistant metal plate, which is 88 - 92 mm in diameter and 0.5 - 1 mm in thickness and has 6 holes with a diameter of 22 - 26 mm can be attached to the top of the upper plastic plate and the bottom of the lower plastic plate. The test apparatus should be attached properly on the motor so that it can move up and down on its central axis.

Auxiliary Disk

Auxiliary disks can be put in a glass column and used only when specified in the monograph. The shape of the disk is cylindrical, with a height of 9.5 ± 0.15 mm and a diameter of 20.7 ± 0.15 mm, and is made of transparent plastic materials with specific gravity of 1.18 to 1.20. There are 5 holes with a diameter of 2 ± 0.1 mm that vertically pierce the top and bottom of the auxiliary disk. One hole is on the center of the auxiliary disk, and the other 4 holes are 6 ± 0.2 mm away from the center at the same interval. Four identical trapezoidal grooves are on the side of auxiliary disk at the same interval, nearly perpendicular to the disk. The trapezoidal shape is symmetrical and its top and bottom parallel lines are parallelized with a line connecting the two adjacent holes, 6 mm from the central axis. The bottom parallel line of the trapezoid has a length of 1.6 ± 1.0 mm and is located at a depth of 1.5 - 1.8 mm from the circumference. The top line is 9.4 ± 0.2 mm long and located at a depth of 2.6 ± 0.1 mm. The disks conform to the dimensions found in Figure 1, and all surfaces of the disks are smooth. When it is permitted to use the auxiliary disk, add a disk to each glass column, and operate the test apparatus as directed in the Procedure. When using specific auxiliary disks processed to automatically detect disintegration, the gravity and size of the disks should comply with the specifications. In addition, they are used only when specified in the monograph.

Auxiliary container

The auxiliary container, as illustrated in Figure 2, consists of a plastic container D, which has an internal diameter of 12 ± 0.2 mm, an outside diameter of 17 ± 0.2 mm, and a length of 20 ± 1 mm, having both outside ends screw-cut, and plastic container A, which has an internal diameter of 12 ± 0.2 mm, an outside diameter of 17 ± 0.2 mm, and a length of 2.5 - 4.5 mm, having one inside end screw-cut. Acid-resistant mesh with a sieve gap of 0.42 mm and a wire diameter of 0.29 mm is placed in each plastic ring, and the rings are attached by screws to each end of the plastic container. The distance between the top and bottom meshes of the auxiliary container is 20 ± 1 mm. A handle of an acid-resistant wire, which is 1 mm in diameter and 80 ± 5 mm in length, is attached to the outer center of the plastic tube by mak-

ing a groove. The auxiliary container is used for the test of granules and capsules containing delayed-release granules.

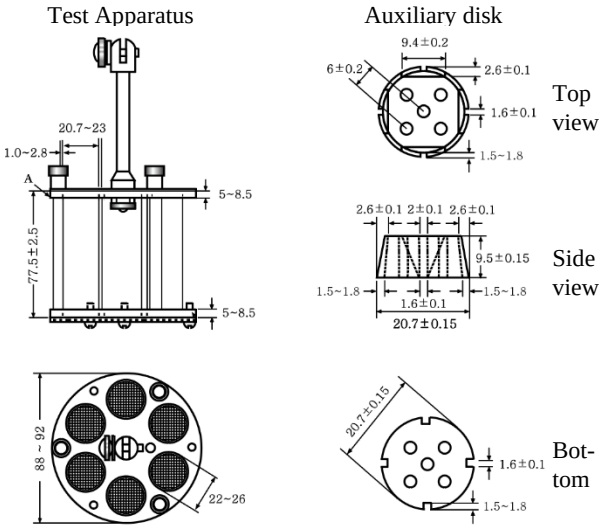


Figure 1. Disintegration Test Equipment

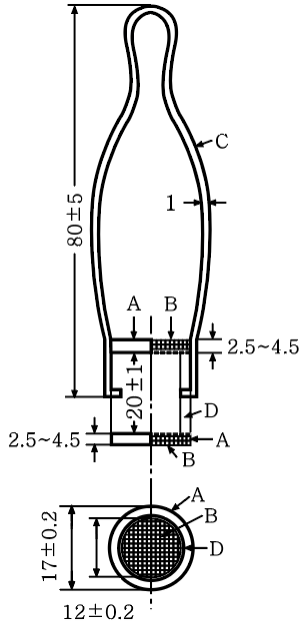


Figure 2. Auxiliary container
A and D; Plastic container
B: 0.42 mm of sieve gap, 0.29 mm of wire diameter
C: Acid-resistant wire handle

Test solution

1) First fluid

Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and add water to make it up to 1000 mL. The fluid is clear and colorless, and its pH is about 1.2.

2) Second fluid

To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS, add 118 mL of 0.2 mol/L sodium hydroxide TS and then add water to make it up to 1000 mL. The fluid is clear and colorless, and its pH is about 6.8.

3) Water

Procedure

1) Immediate-release preparations

For tablets, capsules, and pills (except for pills containing crude drugs), put 1 dosage each of the sample in 6 glass columns of the test apparatus, and if the use of an auxiliary disk is specified, add an auxiliary disk. Unless otherwise specified, run the apparatus at 37 ± 2 °C, using water as the test solution. If the sample floats on the test solution, a flat stainless steel mesh with a sieve gap of 0.57 to 0.66 mm and wire diameter of 1.8 to 2.2 mm may be attached to the upper side of plastic plate on the test apparatus. Unless otherwise specified, remove the tester from the test solution and check the disintegration status 30 minutes after initiating the test for tablets, 60 minutes after for coated tablets and pills, and 20 minutes after for capsules. The result meets the requirements if all of the samples have disintegrated completely. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The result meets the requirements if at least 16 of the 18 samples in total have disintegrated. For pills containing herbal drugs, perform the test for 60 minutes using the same method, with the first fluid used as the test solution. If any residue of the sample is left in the glass column, perform the test successively for 60 minutes, using the second fluid.

For granules, shake granules on a No. 30 (500 μm) sieve as directed in (1) Granules under the Particle Size Distribution Test for Preparations, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary containers, tightly secure each container to the bottom of each test apparatus, and operate the apparatus, using water maintained at 37 ± 2 °C as the immersion fluid, unless otherwise specified. Unless otherwise specified, lift the tester from the test liquid and take out the auxiliary container, and check the disintegration status 30 minutes after initiating the test for plain granules, and 60 minutes after for coated granules. The result meets the requirements if all of the samples have disintegrated completely in an auxiliary container. If 1 or 2 samples in the auxiliary container fail to disintegrate, repeat the test with 12 samples again. The result meets the requirements if at least 16 of 18 samples in total have disintegrated.

2) Delayed-release formulations

Unless otherwise specified, perform the tests twice separately using the first fluid and the second fluid.

i) Delayed-release tablets and capsules

(a) Test with the first fluid

Use the first fluid as the test solution and perform the test for 120 minutes according to the operation method for immediate-release preparations. Disintegration is considered to have occurred when the delayed-release tablet and capsule have disintegrated, or when the delayed-release film is ruptured or broken. The result meets the requirements if none of the samples have disintegrated. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The result meets the requirements if at least 16 of 18 samples in total have not disintegrated.

(b) Test with the second fluid

Use the second fluid as the test solution and perform the test for 60 minutes according to the operation method for immediate-release preparations.

ii) Delayed-release granules and capsules containing delayed-release granules

Sieve granules or contents taken out from capsules using a No. 30 (500 μm) sieve as directed in (1) Granules under the Particle Size Distribution Test for Preparations, transfer 0.10 g each

of the residue remaining on the sieve to each of the 6 auxiliary containers, and insert and secure one container at a time into the glass column of the test apparatus. Then perform 2 tests: A. the test with the first fluid and B. the test with the second fluid.

(a) Test with the first fluid

According to the procedure described for immediate-release preparations, perform the test for 60 minutes, using the first fluid as the test solution. The result meets the requirements if the number of granules fallen from the mess of the test apparatus is NMT 15.

(b) Test with the second fluid

According to the procedure described for immediate-release preparations, perform the test for 30 minutes using the second fluid as the test solution and determine whether the sample has disintegrated.

3) Buccal tablets, sublingual tablets, soluble tablets, dispersible tablets, orally disintegrating tablet (ODT) and chewable tablets

Perform the test according to tablet preparations under the immediate-release procedure, and examine the disintegration status at the time specified in the monograph.

4) Effervescent tablets

Add 1 sample to each of 6 beakers containing 200 mL of water. The nominal volume of a suitable beaker is 250 to 400 mL.

After 5 minutes of the formation of numerous bubbles, or at the time specified in the monograph, the formation of bubbles ceases around each tablet or piece thereof. Each tablet dissolves or disintegrates and disperses in water with no visible coagulation.

5) Effervescent granules

Add 1 unit-dose sample to each of six beakers containing 200 mL of water. The nominal volume of a suitable beaker is 250 to 400 mL.

After 5 minutes of the formation of numerous bubbles, or at the time specified in the monograph, the formation of bubbles ceases around the granules in each beaker. The granules dissolve or disintegrate and disperse in water.

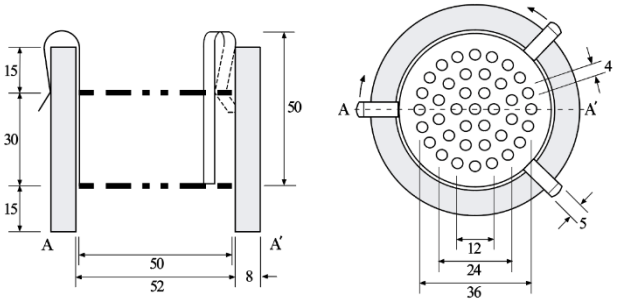
6) Suppositories apparatus

i) As shown in Figure 3, the apparatus consists of a glass or plastic sleeve with the internal diameter of 52 mm, the height of 60 mm, and a thickness of 8 mm.

ii) **Disk** Connect the following device to the sleeve: a device consisting of 2 stainless steel disks, each having 39 holes (4 mm diameter) at the top and bottom of the device. The diameter of the device is similar to that of the interior of the sleeve and the distance between the top and the bottom disks is 30 mm, and 3 metal hooks are attached to the top of the sleeve at equal intervals along the disk circumference.

Procedure

Using water as the test solution, place a suppository on the bottom disk and attach it to the sleeve. Add the apparatus one by one to 3 containers with a temperature of approximately 36 ℃ and a minimum capacity of 4000 mL, or all at once if the container capacity exceeds 12 L. While allowing the water to move slowly, fix the apparatus 90 mm below the surface of the water and invert it every 10 minutes, ensuring it does not float above the water's surface. Unless otherwise specified, fat-based suppositories should soften or disintegrate within 30 minutes and water-soluble suppositories should do so within 60 minutes. The result meets the requirements if the following conditions are met: i) completely dissolved ii) if the components are dissolved, melted fat components gather on the surface of solution, insoluble powders fall to the bottom, and soluble components dissolve. iii) components have a significant change in shape instead of being completely separated or no solid core shows resistance when pressure is applied with a glass rod.



* The figures are in mm.

Figure 3. Suppositories Apparatus

Dissolution
용출시험법

The Dissolution is performed to determine whether an oral preparation is suitable for dissolution specifications. The number of samples used for this test is equivalent to the minimum dose, and unless otherwise specified, this means 1 tablet for tablets, 1 capsule for capsules, and the prescribed amount for other preparations.

1. Apparatus

1) Method 1 (Rotatory basket method, Apparatus 1)

The apparatus consists of a vessel, which may be covered, and is made of glass or another chemically inert[1)] transparent material, a motor, a metal drive shaft, and a cylindrical basket. The vessel is immersed in a suitable constant-temperature water bath of an appropriate size or heated by a suitable device such as a heating cover. The constant-temperature water bath or heating device allows the temperature inside the vessel to be held at 37 ± 0.5 °C during the test and keeps the bath fluid in constant, smooth motion. Any motion, agitation, or vibration beyond that due to the smoothly rotating stirring element should be prevented in the apparatus and its surrounding environment. During the test, the sample and stirring element should be observable. The vessel is cylindrical with a hemispherical bottom, a capacity of 1000 mL, a height of 160 to 210 mm, and an internal diameter of 98 to 106 mm. Its sides are flanged at the top. To prevent evaporation of the dissolution medium, the vessel should be covered with a lid.[2)] The rotating shaft is positioned so that its axis is NMT 2 mm at any point from the vertical axis of the vessel, and rotates smoothly without causing significant movement or vibration that could affect the result. The rotation speed should be adjusted within ± 4% of the specified speed.

Shaft and basket are fabricated of stainless steel (SUS316) or an equivalent inert material, as shown in Figure 1. Also, a basket with a gold coating about 2.5 μm thick can be used. At the beginning of each test, the sample is placed in a dry basket. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

2) Method 2 (Paddle method, Apparatus 2)

Use the same apparatus as Apparatus 1, except that a paddle formed from a stirring blade and a rotation shaft is used as a stirring element. The rotating shaft is positioned so that its axis is NMT 2mm at any point from the vertical axis of the vessel and rotates smoothly without causing significant movement or vibration that could affect the results. The specifications of the paddle are shown in Figure 2, the vertical axis of the blade should pass through the center of the rotation shaft, and the lower part of the blade should be placed on the same plane as the bottom end of the shaft. During the test, the distance between the bottom of the blade and the inside bottom of the vessel is maintained at 25 ± 2 mm. The metallic or suitably inert rigid blade and shaft comprise a single entity. A two-piece detachable design may be used provided that the blade and shaft remain firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating material to make them inert. The sample is allowed to sink to the bottom of the vessel before rotation of the blade is started. A sinker can be used if the sample tends to float during insertion or testing. The sinker is made of chemically inert material, wound several times in a spiral shape, and can be used in the shape shown in Figure 2a. Also, other validated sinkers may be used. When a sinker to use is specified, the sinker shall be the same as in Figure 2a unless otherwise specified.

3) Method 3 (Reciprocating cylinder, Apparatus 3)

The apparatus consists of a set of glass reciprocating cylinders made up of cylindrical, flat-bottomed glass vessels, inert fittings (type 316 stainless steel or other suitable material), sieves that are made of suitable nonadsorbing and nonreactive material and that are designed to fit the tops and bottoms of the reciprocating cylinders, and a motor and drive assembly to reciprocate the cylinders vertically inside the vessels. If necessary, the reciprocating cylinder can also be aligned horizontally with a different row of vessels. During the test, the vessel is partially immersed on a water bath of a suitable size to maintain the temperature at 37 ± 0.5 °C. Any motion, agitation, or vibration beyond that of the reciprocating cylinder should be prevented in the part of the apparatus, including the environment in which the apparatus is placed. The reciprocation should be maintained at the specified rate with a tolerance of ± 5%. It is recommended to use an apparatus that allows observation of the samples and the reciprocating cylinder. Use a vessel that can be covered and keep it covered during the test so that the dissolution medium does not evaporate. Unless otherwise specified, the apparatus must conform to the dimensions shown in Figure 3.

4) Method 4 (Flow-through cell method, Apparatus 4)

The apparatus consists of a reservoir, a delivery pump, a flow-through cell, and constant-temperature water bath that keeps the temperature of the dissolution medium at 37 ± 0.5 °C. The flow-through cell with the size specified in the monograph should be used.

The delivery pump forces the dissolution medium upwards through the flow-through cell. The delivery rate of the delivery pump is 240 to 960 mL per hour, and pumps with standard flow rates of 4 mL/min, 8 mL/min, or 16 mL/min should be used. It must deliver a constant flow (± 5% of the indicated flow rate), and the pulsation wave is sinusoidal, with 120 ± 10 pulses per minute. A pump without pulsation may also be used. Test procedures for dissolution using the flow-through cell method must be characterized with respect to rate and pulsation.

The flow-through cell (see Figures 4 and 5) is made of transparent and chemically inert material and is installed vertically. A filter system (specified in the monograph), which prevents the loss of undissolved particles from the top of the cell, is also mounted. Standard cell diameters are 12 mm and 22.6 mm. The bottom cone is usually filled with small glass beads about 1 mm in diameter, with a bead of about 5 mm positioned at the apex to protect the entry port of the dissolution medium. For special dosage forms, a tablet holder (see Figures 4 and 5) can be used for keeping the sample. The cell is immersed in a constant-temperature water bath to maintain the temperature at 37 ± 0.5 °C.

To prevent leakage, 2 O-rings are used to securely fix the flow-through cell. The delivery pump is installed separately from the dissolution unit to shield the latter against any vibrations originating from the pump. The delivery pump should not be positioned at a level higher than the reservoir flask. Tube connections should be as short as possible and made of a chemically inert material such as polytetrafluoroethylene. It has an internal diameter of approximately 1.6 mm, and chemically inert, flanged-end connections.

2. Apparatus suitability

The determination of suitability of the apparatus to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus given above. In addition, critical test parameters that require regular monitoring during use include volume, temperature, rotation speed of the dissolution medium (in rotary basket method and paddle method), reciprocation rate (in reciprocating cylinder method), and flow rate of dissolution medium (in flow-through cell method).

Regularly determine whether the performance of the apparatus for dissolution test is acceptable.

1) Dissolution medium

A) Medium 1

Dissolve 7.0 mL of hydrochloric acid and 2.0 g of sodium chloride in water to make 1000 mL. This solution is colorless and transparent, and its pH is approximately 1.2.

B) Medium 2

A mixture of phosphate buffer solution, pH 6.8 and water (1:1)

3. Procedure

1) Method 1 and Method 2

A) Immediate-release preparation

(i) ***Procedure*** Place the specified volume of the dissolution medium (± 1%) in the vessel of the specified apparatus. Equilibrate the temperature of the dissolution medium at 37 ± 0.5 °C, and remove the thermometer. Place one dosage unit of the preparation in the vessel, ensuring that there are no air bubbles from the surface of the sample, and immediately operate the apparatus at the specified rotation speed. Within the interval or duration specified, collect a sample from a zone midway between the surface of the test solution and the top of the rotary sample basket or blade, NLT 10 mm from the container wall. (Note: Where no less than 2 times of dissolution medium sampling is specified, replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium at 37 °C, or if replenishment of the dissolution medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered during the test and verify the temperature of the dissolution medium in the vessel at suitable times.) Measure the amount of eluted active pharmaceutical ingredient using a suitable assay method.[3)] Repeat this test with additional samples.

If automated equipment is used for sampling or the apparatus is otherwise modified, ensure that the modified apparatus produces results equivalent to those obtained with the apparatus

described in the general test.

(ii) *Dissolution medium* Proceed by using specified dissolution medium. The specified volume is measured at between 20 °C and 25 °C. If the dissolution medium is a buffered solution, adjust its pH to be within ± 0.05 of the specified pH. (Note: Dissolved gases can cause bubbles to form, which may change the results of the test. If dissolved gases influence the dissolution results, dissolved gases must be removed prior to testing[4]).

(iii) *Duration* Where a single time point is specified, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. In other cases, specimens are to be withdrawn only at the stated times within a tolerance of ± 2%.

B) Extended-release preparations

(i) *Procedure* Proceed as described for immediate-release preparations.

(ii) *Dissolution medium* Proceed as described for immediate-release preparations.

(iii) *Duration* Generally, the result is measured at 3 time points and expressed in hours.

C) Delayed-release preparations

(i) *Procedure* Proceed as directed under Method (A) or (B). Collect the dissolution medium within ± 2% of the specified time unless otherwise specified.

A) Method (A)

Acid stage

Add 750 mL of 0.1 mol/L hydrochloric acid TS to the vessel and assemble the apparatus. Maintain the dissolution medium at a temperature of 37 ± 0.5 °C. Add 1 sample to the apparatus, cover the vessel, and operate the apparatus at the specified rate. After performing the test for 2 hours in 0.1 mol/L hydrochloric acid TS, withdraw an aliquot of the dissolution medium and proceed immediately according to the procedure directed under the Buffer stage. Measure the amount of the active ingredient eluted using the specified assay.

Buffer stage

While operating the apparatus at the specified rotational rate, add 250 mL of 0.2 mol/L sodium phosphate solution equilibrated to a temperature of 37 ± 0.5 °C to 0.1 mol/L hydrochloric acid TS above. If necessary, adjust the pH of the medium to 6.8 ± 0.05 using 2 mol/L hydrochloric acid TS or 2 mol/L sodium hydroxide TS (Complete the operations of adding 0.2 mol/L sodium phosphate solution and adjusting the pH within 5 min).

Operate the apparatus for 45 minutes or for the time specified in the monograph and collect the dissolution medium. Measure the amount of the active ingredient eluted in the collected medium using the specified assay.

B) Method (B)

Acid stage

Add 1000 mL of 0.1 mol/L hydrochloric acid TS to the vessel and assemble the apparatus. Maintain the dissolution medium at a temperature of 37 ± 0.5 °C. Add 1 sample to the apparatus, cover the vessel, and operate the apparatus at the specified rotational rate. After performing the test for 2 hours in 0.1 mol/L hydrochloric acid TS, remove an aliquot of the dissolution medium and proceed immediately according to the procedure directed under the Buffer stage. Measure the amount of the active ingredient eluted using the specified assay.

Buffer stage

Use a buffer solution equilibrated to a temperature of 37 ± 0.5 °C. Remove the dissolution medium collected in the Acid stage and add 1000 mL of pH 6.8 phosphate buffer solution, prepared by mixing 0.1 mol/L hydrochloric acid TS with 0.20 mol/L sodium phosphate (3 : 1). If necessary, adjust the pH to 6.8 ± 0.05 with 2 mol/L hydrochloric acid or 1 mol/L sodium hydroxide. [Note: This may also be accomplished by removing from the apparatus the vessel containing the 0.1 mol/L hydrochloric acid, then replacing it with another vessel containing the buffer solution, and transferring the sample to the vessel containing the buffer solution. The sample removed from the vessel containing 0.1 mol/L hydrochloric acid and the paddle or rotating basket can be used in a vessel for the pH 6.8 phosphate buffer solution.]

Operate the apparatus for 45 minutes or for the time specified in the monograph and collect the dissolution medium. Measure the amount of the active ingredient eluted in the collected medium using the specified assay.

2) Method 3

A) Immediate-release preparation

(i) *Procedure* Place the specified volume of the dissolution medium in the vessel of the specified apparatus. Equilibrate the temperature of the dissolution medium at 37 ± 0.5 °C, and remove the thermometer. Place 1 sample in each of the reciprocating cylinders, making sure that there are no air bubbles on the surface of each sample, and immediately operate the apparatus according to the conditions directed in the monograph. During the upward and downward strokes, the reciprocating cylinder moves a total distance of 9.9 to 10.1 cm. Within the specified time interval or at each of the specified times, raise the reciprocating cylinders and collect a portion of the medium from an area midway between the surface of the dissolution medium and the bottom of each vessel. Perform the test according to the conditions directed in the monograph. If necessary, repeat this test with additional samples.

(ii) *Dissolution medium* Proceed as directed for immediate-release preparations under Method 1 and Method 2.

(ii) *Duration* Proceed as directed for immediate-release preparations under Method 1 and Method 2.

B) Extended-release preparations

(i) *Procedure* Proceed as directed for immediate-release preparations under Method 3.

(ii) *Dissolution medium* Proceed as directed for extended-release preparations under Method 1 and Method 2.

(iii) *Duration* Proceed as directed for immediate-release preparations under Method 1 and Method 2.

C) Delayed-release preparations

(i) *Procedure* Proceed as directed for delayed-release preparations (B) under Method 1 and Method 2.

(ii) *Dissolution medium* Use one row of vessels for the dissolution medium in the Acid stage and the following row of vessels for the dissolution medium in the Buffer stage. Use the specified volume of the dissolution medium (usually 300 mL).

(iii) *Duration* Proceed as directed for immediate-release preparations under Method 1 and Method 2.

3) Method 4

A) Immediate-release preparation

(i) *Procedure* Fill the glass beads into the cell specified in the monograph. Place 1 sample on top of the beads or on a wire rack. Assemble the filter head and fix the parts together using a suitable clamp. Using the pump, introduce the test solution heated to 37 ± 0.5 °C into the cell from the bottom of the flow-through cell at a flow rate within ± 5% tolerance of the specified value. Collect the eluate at each specified interval. Measure the

amount of the active ingredient eluted using the specified assay. Repeat this test with additional samples.

(ii) *Dissolution medium* Proceed as described for immediate-release preparations under Method 1 and Method 2.

(iii) *Duration* Proceed as described for immediate-release preparations under Method 1 and Method 2.

B) Extended-release preparations

(i) *Procedure* Proceed as described for immediate-release preparations under Method 4.

(ii) *Dissolution medium* Proceed as described for immediate-release preparations under Method 4.

(iii) *Duration* Proceed as directed for immediate-release preparations under Method 4.

C) Delayed-release preparations

(i) *Procedure* Proceed as directed for delayed-release preparations under Method 1 and Method 2. Use the specified dissolution medium.

(ii) *Duration* Proceed as directed for delayed-release preparations under Method 1 and Method 2.

4. Interpretation

1) Immediate-release preparation

Follow Interpretation method 1 when the value Q is specified in the monograph in other cases, follow Interpretation method 2.

A) Interpretation method 1

Unless otherwise specified, it is considered suitable if the dissolution rate of the active ingredient from the sample tested conforms to Interpretation Table 1. Continue testing through S_3 unless the results at either S_1 or S_2 conform. Q represents the specified dissolution rate of the active ingredient, expressed as a percentage of the labeled content of the sample. In Table 1, 5%, 15%, and 25% values are percentages of the labeled content of the active ingredient, as indicated for Q.

Table 1. Interpretation table

Level	Number tested	Acceptance criteria
S_1	6	Each sample value is no less than Q + 5%
S_2	6	Average value of the 12 samples $(S_1 + S_2) \geq Q$, and no sample is less than Q − 15%.
S_3	12	Average value of the 24 samples $(S_1 + S_2 + S_3) \geq Q$, NMT 2 samples are less than Q − 15%, and no sample is less than Q − 25%.

B) Interpretation method 2

Unless otherwise specified, perform the test on 6 samples. It is considered suitable if the dissolution rate of each sample is a value specified in the monograph. If 1 or 2 sample(s) fail to meet the specified value requirements, repeat the test on 6 new samples. The result is considered suitable if individual dissolution rates of 10 or more out of the 12 samples are consistent with the specified value requirements.

2) Extended-release preparations

A) Interpretation method 1

Unless otherwise specified, it is considered suitable if the dissolution rate of the active ingredient in a sample tested conform to Interpretation Table 2. Continue testing through L_3 unless the results at either L_1 or L_2 conform. The limits of the dissolution rate at each time point is shown are the percentage of labeled content. The limits embrace each value of Q_i, the dissolution rate at each specified fractional dosing interval. Where more than one range is specified in the monograph, the acceptance criteria apply individually to each range.

Table 2. Interpretation table

Level	Number tested	Acceptance criteria
L_1	6	All individual sample values are within their specified ranges (including the limit values), and at the final test time, no individual value is less than the specified one.
L_2	6	The average value of the 12 samples $(L_1 + L_2)$ is within the specified range and is NLT the specified value at the final test time. No value is more than 10% of the labeled content outside each of the specified ranges and no value is more than 10% of the labeled content below the specified value at the final test time.
L_3	12	The average value of the 24 samples $(L_1 + L_2 + L_3)$ is within the specified range and is NLT the specified value at the final test time. NMT 2 of the 24 values are more than 10% of the labeled content outside each of the specified ranges; NMT 2 of the 24 values are more than 10% of the labeled content below the specified value at the final test time. None of the samples is more than 20% of the labeled content outside each of the specified ranges or more than 20% of the labeled content below the specified value at the final test time.

B) Interpretation method 2

Unless otherwise specified, perform the test on 6 samples. It is considered suitable if the dissolution rate of each sample is a value specified in the monograph. If 1 or 2 sample(s) fail to meet the specified value requirements, repeat the test on another 6 samples. The result is considered suitable if individual dissolution rates of 10 or more out of the 12 samples are consistent with the specified value requirements. Where more than one range is specified, the acceptance criteria apply individually to each range.

3) Delayed-release preparations

If the Q value is specified for tests using the second fluid for the Dissolution in the monograph, follow Interpretation Method 1, and in other cases, follow Interpretation Method 2.

A) Interpretation method 1

(i) *Test using the first fluid for the Dissolution* Unless otherwise specified, in a test using the first fluid for the Dissolution, it is considered suitable if the dissolution rate of the active ingredient conforms to Interpretation Table 3. If the test results from the first fluid and the second fluid do not conform to the baseline level, perform the test at all levels.

Table 3. Interpretation table

Level	Number tested	Acceptance criteria
A_1	6	Each sample value is less than 10%.
A_2	6	The average value of 12 samples $(A_1 + A_2)$ is less than 10% and each value is not greater than 25%.
A_3	12	The average value of 24 samples $(A_1 + A_2 + A_3)$ is less than 10% and each value is not greater than 25%.

(ii) ***Test using the second fluid for the Dissolution*** Unless otherwise specified, it is considered suitable if the dissolution rate of the active ingredient conforms to Table 4. Continue testing through B_3 unless the results conform at either B_1 or B_2. Unless otherwise specified, the Q value in Table 4 represents a dissolution rate of 75%. The Q value specified in the monograph is the total amount of active substance eluted in both the Acid stage and the Buffer stage, and is expressed as a percentage relative to the labeled amount. In Table 4, the 5%, 15%, and 25% values are percentages of the labeled content of the active ingredient, as indicated for Q.

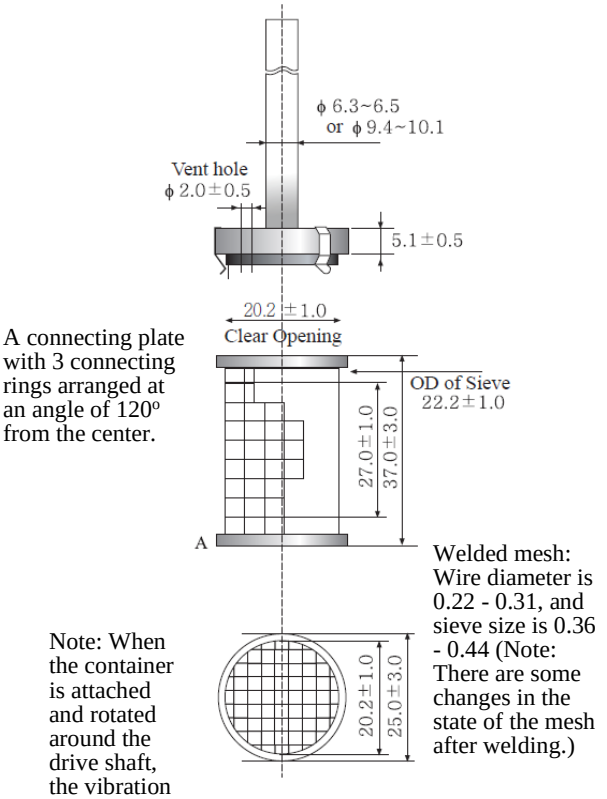
Table 4. Interpretation table

Level	Number tested	Acceptance criteria
B_1	6	Each sample value is more than Q + 5%.
B_2	6	Average of 12 samples ($B_1 + B_2$) ≥ Q, and no sample is less than Q − 15%.
B_3	12	Average of 24 samples ($B_1 + B_2 + B_3$) ≥ Q, NMT 2 samples are less than Q − 15%, and no sample is less than Q − 25%.

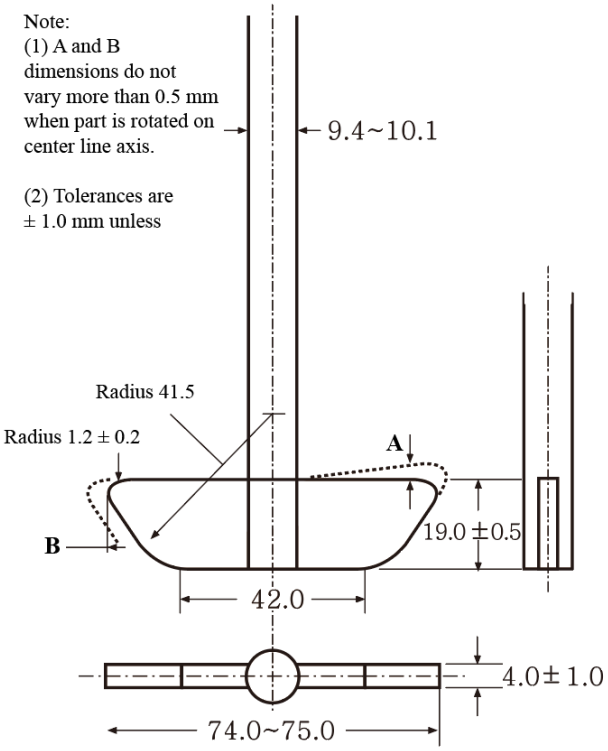
B) Interpretation method 2

Unless otherwise specified, in using the first fluid and the second fluid for the Dissolution test, it is considered suitable if the dissolution rate of each sample meets the value requirements specified in the monograph in a test performed on 6 samples. If 1 or 2 sample(s) fail to meet the specified value requirements, repeat the test on 6 new samples. The result is considered suitable if the individual dissolution rates of 10 or more out of the 12 samples are consistent with the specified value requirements.

1) The materials should not adsorb, react with samples, or interfere with the testing of samples.
2) If a cover is used, the opening should be secured in advance to ensure that the thermometer and a tool for sampling can be inserted.
3) Samples are filtered immediately upon sampling unless filtration is unnecessary. Use a filter that does not absorb the active ingredient and does not contain extractable substances that would interfere with the analysis.
4) A method of degassing is as follows: heat the medium to about 41 °C while stirring gently, immediately filter using a filter with a pore size of 0.45 μm or less, with suction and stirring, and continue stirring under vacuum for about 5 min. Other validated degassing techniques for removal of dissolved gases may be used.

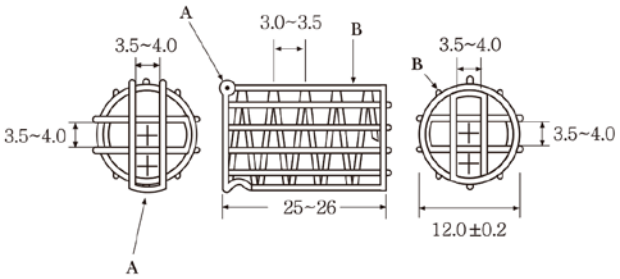


Ⅰ. Apparatus 1, Drive shaft and container



* The figures are in mm.

Figure 2. Apparatus 2, Drive shaft and stirring blade



* The figures are in mm.

Figure 2a. Sinker specification

A: Acid-resistant lock
B: Acid-resistant

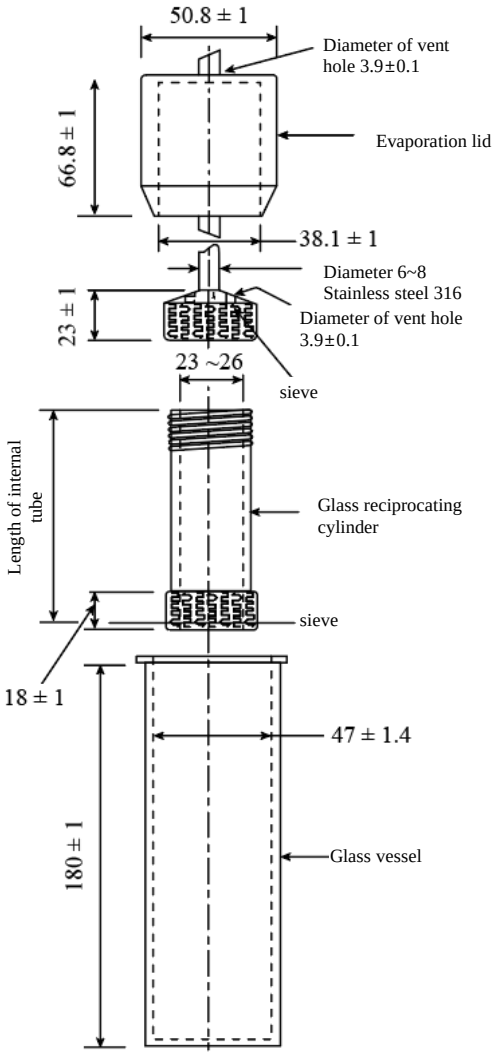
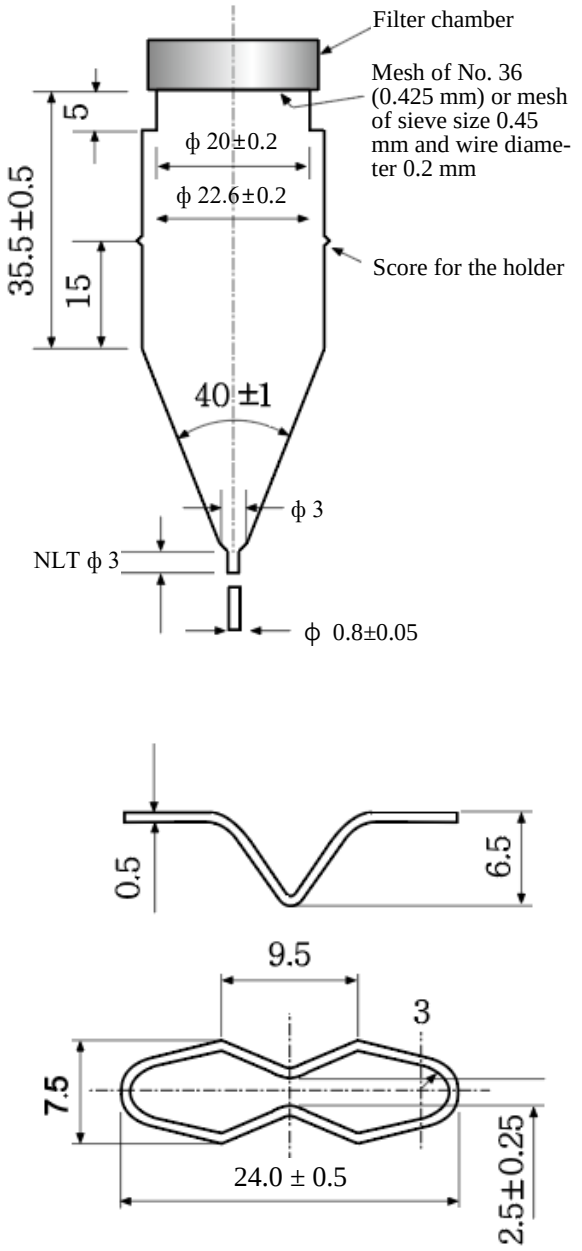
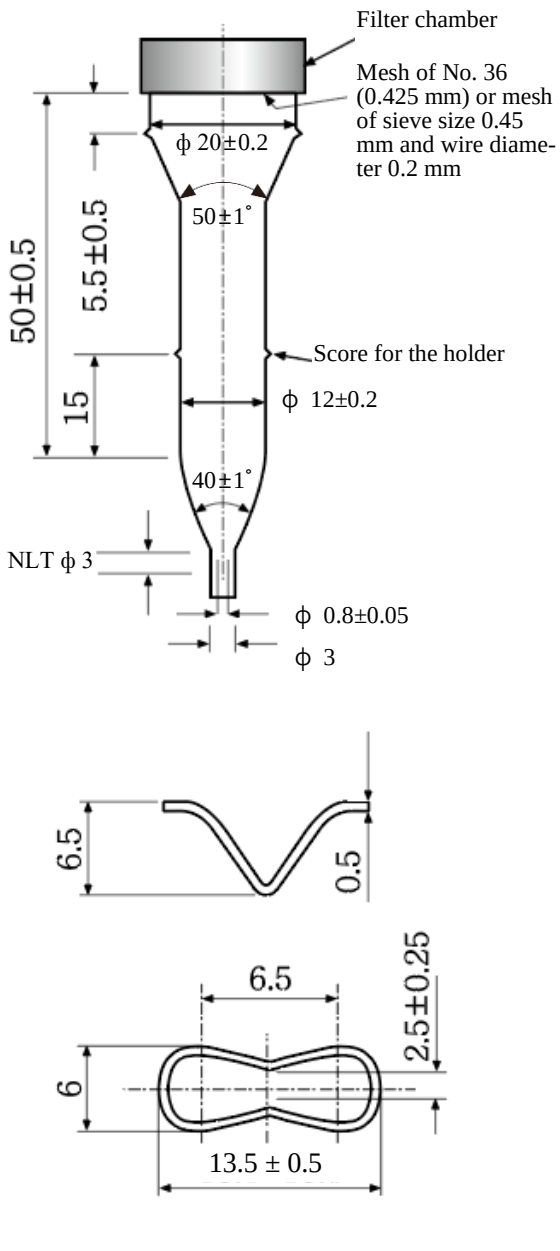


Figure 3. Apparatus 3 Reciprocating cylinder



Numbers are indicated in mm.
ϕ are diameters.

Figure 4. Apparatus 4
(Top) Large flow through cell for tablets and capsules
(Bottom) Tablet holder for the small flow through cell
(Lengths are shown in mm unless otherwise specified.)



Numbers are indicated in mm.
ϕ are diameters.

Figure 5. Apparatus 4
(Top) Small flow through cell for tablets and capsules
(Bottom) Tablet holder for the large flow through cell
(Lengths are shown in mm unless otherwise specified.)

Elastomeric Closures for Injections
주사제용고무마개시험법

Elastomeric closures for injections refer to rubber stoppers (including those coated or laminated with materials such as plastic, etc.) used to close containers for injections. The rubber closure should not physically or chemically interact with the drug contained therein to affect its description and quality. It should prevent the invasion of microorganisms, and not interfere with the functionality of injections.

1) Eluate testing

Wash the rubber closure with water and dry it at ordinary temperature. Take a sample with a surface area of approximately 100 ± 10 cm^2 (if necessary, take several samples with a surface area close to 100 cm^2) and place the whole in a hard glass container. Add purified water or water for injection to make 2 mL per 1 cm^2 of the sample, and weigh it. Place a stopper in a non-reactive container made of hard glass or similar, and heat up to 121 ± 2 °C in an autoclave within 20 to 30 minutes, and maintain this temperature for 30 minutes. At this time, the container containing water and the temperature probe is placed in the autoclave with the sample to control the temperature. Take out the sample container, and leave it for about 30 minutes until it reaches ordinary temperature. Add purified water or water for injection to return to the original mass, shake and decant the sample immediately, and use the resulting solution as the test solution. Separately, prepare a blank test solution in the same way with 200 mL of purified water or water for injection. Perform the following tests with the sample and blank test solutions. However, the sample and control solution in G) volatile sulfides are prepared separately.

A) Turbidity

Perform the test by comparing with the naked eye or using the Photoelectric Photometry method according to the Turbidimetry.

Method 1 Visual comparison

When tested according to visual comparison of the Turbidimetry, the turbidity of the test solution is not darker than the turbidity control solution II. However, when tested with a rubber closure for multiple use, the turbidity of the test solution is not darker than the turbidity control solution III.

Method 2 Photoelectric Photometry

Measure the turbidity of the turbidity control solutions using a properly calibrated turbidimeter according to the Photoelectric Photometry of the Turbidity. When the blank test solution is measured and calibrated in the same manner, the turbidity control solution I, turbidity control solution II, turbidity control solution III, and turbidity control solution IV show 3, 6, 18, and 30 NTU (Nephelometric Turbidity Units), respectively. The turbidity of the test solution corrected with the turbidity of the blank test solution using a calibrated turbidimeter is less than 6 NTU. When testing with a rubber closure for multiple use, the turbidity of the test solution corrected with the blank test solution is less than 18 NTU.

B) Color of the solution

Add 97 mL of dilute hydrochloric acid to 3 mL of the color-comparison solution O, and use it as the standard solution. Use the same test tube made of a colorless, transparent test tube made of non-reactive material with a flat bottom and an internal diameter of 15 to 25 mm. Add the test solution to a depth of 40 mm on one side. On the other side, add the color standard solution to the same depth. Observe and compare the colors of the solutions vertically under bright light, avoiding direct sunlight and using a white background; the color of the test solution is not darker than the standard solution.

C) Acidity or alkalinity

When adding 0.1 mL of bromothymol blue solution to 20 mL of acid or alkali test solution. If it turns yellow, titrate with 0.01 mol/L sodium hydroxide solution until it appears blue. When the blue color appears, titrate with 0.01 mol/L hydrochloric acid until the yellow color appears again. However, if the color of the solution is green, it means that it is neutral and titration is not necessary. Titrate the blank test solution in the same way, and make any necessary correction. It is suitable if 0.01 mol/L sodium hydroxide solution used to produce a blue color is less than 0.3 mL, or 0.01 mol/L hydrochloric acid consumed to produce a yellow color is 0.8 mL, or if there is no need for titration.

Bromothymol blue solution: Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 mol/L sodium hydroxide solution and 20 mL of ethanol (96) and add water to make 100 mL.

D) Reducing substances

Perform this test within 4 hours of preparing the test solution. Add 1 mL of dilute sulfuric acid and 20.0 mL of 0.002 mol/L potassium permanganate solution to 20.0 mL of the test solution and boil for 3 minutes. After cooling, add 1 g of potassium iodide, immediately add 0.25 mL of starch TS as an indicator, and titrate with 0.01 mol/L sodium thiosulfate solution. Titrate the blank test solution in the same manner. The difference in the consumption of 0.01 mol/L sodium thiosulfate solution between the sample and blank test solutions is less than 3.0 mL. When testing with a rubber closure for multiple use, the difference in consumption of 0.01 mol/L sodium thiosulfate solution is less than 7.0 mL.

E) Ultraviolet-visible absorption spectrum

Perform this test within 5 hours of preparing the test solution. Filter the test solution through an inert filter with a pore size of 0.45 μm, discard the first few mL, and use the next filtrate. The absorbance of the filtrate at a wavelength of 220 to 360 nm is less than 0.2 when tested according to the Ultraviolet-visible Spectrophotometry with 1 cm cell using the blank test solution as the blank. When tested with a rubber closure for multiple use, the absorbance is less than 4.0. When the filtrate is diluted and measured, it is corrected by the dilution factor.

F) Ammonium

Add water to 5 mL of the test solution to make 14 mL. If necessary, add 1 mol/L sodium hydroxide TS to make it alkaline and add water to make 15 mL. Add 0.3 mL of alkaline potassium tetraiodomercurate(II) solution, close the container, and use it as the test solution. Separately, add 5 mL of water to 10 mL of ammonium chloride solution [1 ppm of ammonium ion (NH_4^+)], add 0.3 mL of alkaline potassium tetraiodomercurate(II) solution, close the container, and use it as the blank. Let the sample and control solutions stand for 5 minutes. At this time, the yellow color of the test solution is not darker than the color of the control solution [2 ppm of ammonium ion (NH_4^+) in the test solution].

Alkaline potassium tetraiodomercurate(II) solution: Dissolve 11 g of potassium iodide and 15 g of mercury(II) iodide in water to make 100 mL. Mix equal volumes of the resulting solution and 25% sodium hydroxide solution just before use.

G) Volatile sulfides

Ensure that the total surface area of the rubber closure is 20 ± 2 cm^2, if necessary, cut the rubber closure, and then place it in a 100 mL flask. Add 50 mL of 2 w/v% citric acid solution, and use it as the sample. Simultaneously, dissolve 0.154 mg of sodium sulfide in 50 mL of 2 w/v% citric acid solution in the same manner, and use it as the blank. Place a piece of lead acetate paper on the opening of each flask containing the sample and control solution and place the weighing bottle upside down on top of it to fix the lead acetate paper. Separately, add 50 mL of water to a flask similar to the one used for the sample and immerse the temperature probe for program control of the autoclave in it. Heat with an autoclave up to 121 ± 2℃ within 20 to 30 minutes and maintain this temperature for 30 minutes. Cool down the sample to ordinary temperature for about 30 minutes. At this time, the black stain on the lead acetate paper produced by the sample is not darker than that of the control solution.

2) Biological reactivity tests

The following tests are used to determine the toxicity of the samples following contact with the elastomeric plastics and other polymeric materials and include *in vitro* and *in vivo* tests. The sample must be suitable for in vitro testing. If not, in vivo testing will be performed to make the final determination.

A) *In vitro* tests

This test must be performed on a specified surface. However, if the surface area of the sample cannot be measured, 0.1 g of sample per 1 mL is used. Perform the test according to the appropriate method among the following methods, taking into consideration the characteristics of the sample, etc.

Test methods

Test control material

(1) Negative control material

High-density polyethylene reference standard

(2) Positive control material

Polyurethane film containing 0.1% zinc diethyldithiocarbamate or 0.25% zinc dibutyldithiocarbamate

Cell culture preparation

The cell line used is L-929 (ATCC cell line CCl 1, NCTC clone 929; alternative cell lines obtained from a standard repository may be used with appropriate validation) mammalian fibroblast cells. The medium used is Eagle's minimum essential medium with added bovine serum (10 vol%), which is used for cytotoxicity test of the Plastic Containers for Pharmaceutical Use. Subculture the cell lines at a density of approximately 10^5 per mL of this medium. Incubate the culture at 36 to 38℃ in a humidified incubator for at least 24 hours with a carbon dioxide concentration of 4 to 6% until differentiation exceeds 80%. Observe the cultured cells under a microscope to ensure that the monolayers are uniform and cover almost the entire surface. [Note: The reproducibility of the in vitro biological reactivity tests depends on the uniform density of the cell culture medium]

Extraction solvent

Use sodium chloride injection (sodium chloride injection containing 0.9% sodium chloride in the monograph) as the extraction solvent. Alternatively, serum-free mammalian cell culture medium or serum-supplemented mammalian cell culture medium may be used. Serum supplementation is used when extraction is performed at 37° for 24 hours.

Apparatus

Autoclave Use an autoclave that can maintain a temperature of 121 ± 2℃ and is equipped with a thermometer, a pressure gauge, a vent cock, a rack that can hold the test containers above water, and a cooling system that can cool the test containers to about 20 ℃. However, when cooling down after sterilization, make sure that the temperature does not drop below 20℃.

Thermostat

Use a thermostat, preferably a mechanical convection model with an accuracy of ± 2 ℃, that maintains the operating temperature at 50 - 70℃.

Incubator

Use an incubator that can maintain a temperature of 37 ± 1℃ and 5 ± 1% carbon dioxide.

Extraction containers

Use only containers such as ampoules or culture test tubes with screw caps or their equivalent made of hard glass. If culture test tubes or their equivalent are used, they should be sealed with a screw cap with a suitable elastomeric liner. The exposed surface of the elastomeric liner is completely covered with an inert solid disk with a thickness of 50 to 75 μm. A suitable disk made of polytef can be used.

Preparation of apparatus

Cleanse all glassware thoroughly with chromic acid cleansing mixture, with heated nitric acid if necessary, followed by a prolonged rinse with sterile water for injection. Sterilize and dry containers and devices used for extraction, transfer, or administration of test materials through a suitable process. If ethylene oxide is used as a sterilizing agent, it should be degassed completely for at least 48 hours.

Procedure

Preparation of sample for extracts

Prepare as directed in B) in vivo tests.

Preparation of extracts

Prepare as directed in the preparation of extracts in the in-vivo tests operation method using either sodium chloride injection (0.9% sodium chloride) or serum-free mammalian cell culture medium as the extraction solvent. [Note: If extraction is performed at 37℃ for 24 hours in an incubator, use serum-supplemented cell culture medium. The extraction conditions should not, under any conditions, cause physical changes, such as fusing or melting of the material pieces, except for a slight adhesion.

Method 1 Agar diffusion test

In this test, the agar layer serves as a cushion that protects the cells from mechanical damage while allowing diffusion of leachable chemicals from the polymeric sample.

Sample preparation

Use extracts prepared as specified, or use the portion of the sample with flat surface of 100 mm^2 or more.

Positive control preparation

Proceed as specified in the sample preparation.

Negative control preparation

Proceed as specified in the sample preparation.

Procedure

Take 7 mL of the cell suspension prepared as described in the cell culture medium preparation and make the monolayer of the cells on 60 mm diameter cell culture plates. After incubation, remove the culture medium from the monolayers, and add a serum-supplemented culture medium containing less than 2% of agar. [Note: The amount of agar must be sufficient to promote cell growth. The agar layer must be thin enough to allow diffusion of the effused chemicals.] Place the flat surface of the sample, positive control, and negative control or their extracts in a suitable extraction medium in contact with the solidified agar surface, and place them in 2 cultures. Use NMT three samples per prepared culture plate. Incubate all cultures for at least 24 hours at 36 to 38℃, preferably in a humidified incubator with a carbon dioxide concentration of 4 to 6%. Perform an appropriate stain if

necessary, and observe cultured cells around each sample, negative control, and positive control using a microscope.

Interpretation of results

Biological reactivity (cellular degeneration and malformation) is expressed and rated on a scale of 0 to 4, as shown in Table 1. Measure the reactivity of the cell culture medium of each sample, negative control, and positive control. The cell culture test system is considered suitable if the observed reactivity to the negative control is grade 0 (no reactivity) and to the positive control is at least grade 3 (moderate). The sample meets the requirements of the test if the reactivity of the prepared sample is less than grade 2 (mild reactivity). Repeat the procedure if the suitability of the system is not confirmed.

Table 1. Reactivity grades for agar diffusion test and direct contact test

Grade	Reactivity	Description of reactivity zone
0	None	No detectable zone around or under the sample
1	Slight reactivity	Some malformed or degenerated cells under the sample
2	Mild reactivity	Malformed or degenerated cells are limited under the sample, and the reactive zone outside the sample is less than 0.45 cm
3	Moderate reactivity	Reactive zone outside the sample extends to 0.45 -1.0 cm
4	Severe reactivity	Reactive zone outside the sample extends to more than 1.0 cm

Method 2 Direct contact test

This test may be not suitable for very low- or high-density materials that could cause mechanical damage to the cells.

Sample preparation

Use portions of the sample with flat surfaces of 100 mm^2 or more.

Positive control preparation

Proceed as specified in the sample preparation.

Negative control preparation

Proceed as specified in the sample preparation.

Procedure

Take 2 mL of the cell suspension prepared as described in the cell culture medium preparation and make the monolayer of the cells on 35 mm diameter cell culture plates. After incubation, remove the medium from the culture medium, and add 0.8 mL of fresh culture medium. Add one sample, a positive control, and a negative control in each of the two culture medium. Incubate all cultures for at least 24 hours at 36 to 38 ℃, preferably in a humidified incubator with a carbon dioxide concentration of 4 to 6%. Perform an appropriate stain if necessary, and observe the surrounding culture of each sample, negative control, and positive control under a microscope or with the naked eye.

Interpretation of results

Proceed as specified in the interpretation of results of the agar diffusion test. The sample meets the requirements of the test if the reactivity of the prepared sample is less than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed.

Method 3 Elution test

This test is suitable for high-density materials and for dose-response evaluations.

Preparation of sample

Prepare as directed in the preparation of extracts using either sodium chloride injection (0.9% sodium chloride) or serum-free mammalian cell culture medium as the extraction solvent. If the surface area of sample cannot be easily measured, a mass of 0.1 g of the rubber closure per mL of extraction medium may be used. Alternatively, use serum-supplemented mammalian cell culture medium as the extracting medium to approximate physiological conditions. The extracts are prepared by incubating for at least 24 hours in a humidified incubator with a carbon dioxide concentration of 4 to 6%. The extraction temperature is maintained at 36 to 38℃ because high temperatures may cause denaturation of serum proteins.

Positive control extract preparation

Proceed as specified in the sample preparation.

Negative control extract preparation

Proceed as specified in the sample preparation. Use high-density polyethylene reference standard as a negative control material.

Procedure

Take 2 mL of the cell suspension prepared as described in the cell culture medium preparation and prepare the monolayers in 35 mm diameter plates. After incubation, remove the culture medium from the monolayers, and add extracts of the sample, positive control, and negative control. For the serum-supplemented and serum-free cell culture medium extracts, test twice without dilution (100%). For the sodium chloride injection extract, test twice by diluting serum-supplemented cell culture medium at 25% extract concentration. Incubate all cultures for 48 hours at 36 to 38℃, preferably in a humidified incubator with a carbon dioxide concentration of 4 to 6%. Perform an appropriate stain if necessary, and observe each culture under a microscope.

Interpretation of results

Proceed as directed in the results interpretation of the agar diffusion test, while referring to Table 2. The sample meets the requirements of the test if the reactivity to the sample is less than grade 2 (mildly reactive). Repeat the procedure if the suitability of the system is not confirmed. For dose-response evaluations, repeat the procedure using quantitative dilutions of the sample extract.

Table 2. Reactivity grades for elution test

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight reactivity	Less than or equal to 20% of the cells are round, loosely attached, no intracytoplasmic granules, and lysed cells present occasionally
2	Mild reactivity	Less than or equal to 50% of the cells are round, no intracytoplasmic granules, and no extensive cell lysis and empty areas between cells
3	Moderate reactivity	Less than or equal to 70% of the cell layers are round or lysed
4	Severe reactivity	Cell layers are almost completely destroyed

B) *In vivo* tests

This test is performed when the sample does not meet the specifications of the in vitro test, and the final decision is made based on the in vivo test results. In this test, a specific surface area for extraction is essential. However, when the surface area of the sample cannot be measured, use 0.1 g of a rubber closure per mL of the extraction solvent. The test is performed by either method 1 or method 2.

For the purpose of this test, the terms are defined as follows:

Sample A test material or an extract prepared from it.

Blank test solution A solution containing the same quantity of the same extracting medium used for the extraction of the sample under test, treated in the same manner as the extracting medium.

Negative control A substance that shows no reaction under the test conditions, such as high-density polyethylene reference standard.

In the procedure, use the extract prepared at one of three standard temperatures, that is 50, 70, and 121℃, depending on the thermal resistance of the material.

Extraction solvent Sodium chloride injection (see monograph). Use sodium chloride injection containing 0.9% sodium chloride.

A mixture of sodium chloride injection/ethanol (19 : 1)

Polyethylene glycol 400 (see monograph)

Vegetable oil Use freshly refined sesame oil (see monograph) or cottonseed oil (see monograph) or other suitable vegetable oils

Drug product vehicle (if applicable)

Water for injection (see monograph)

[Note: The sesame oil or cottonseed oil or other suitable vegetable oil should meet the following additional requirements. Use refined oil as fresh as possible. Use three properly raised animals and inject the oil intracutaneously with a dose of 0.2 mL into each of 10 sites per animal. Observe the animals at 24, 48, and 72 hours after injection. Rate the observations at each site on the numerical scale indicated in Table 3. For the 3 rabbits (30 injection sites) or guinea pigs (18 injection sites), the average response to erythema is less than 0.5 and to edema is less than 1.0 at each observation time. No site shows tissue reactions with a diameter of more than 10 mm. The oil residue at the injection site should not be misinterpreted as edema. Edematous tissue turns white when gentle pressure is applied.

Table 3. Evaluation of skin reactions[1)]

Erythema and eschar formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Obvious erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema formation[2)]	Score
No edema	0
Very slight edema (barely noticeable)	1
Slight edema (edges of edema area are clearly raised)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extended beyond the exposed area)	4

1) Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82:377–390.

2) Excludes non-inflammatory (mechanical) edema from the blank or extraction solvent.

Apparatus The test apparatus includes the following.

Autoclave Use an autoclave as described in A).

Thermostat Use a thermostat as described in A).

Extraction containers Use extraction containers as described in A).

Preparation of the apparatus Cleanse all glassware thoroughly with chromic acid cleansing mixture, with heated nitric acid if necessary, and then washed with water for a long time. Before cutting the specimen, cutting utensils should be cleaned appropriately (e.g., successive washes with acetone and methylene chloride). Clean all other equipment by thoroughly scrubbing with a suitable detergent and prolonged rinsing with water several times.

Render containers and equipment used for extraction, transfer and administration should be sterilized and dried through a suitable process. [Note: If ethylene oxide is used as a sterilizing agent, leave it for an adequate time to completely degas.]

Procedure

Preparation of the sample

Both the systemic injection test and the intracutaneous test may be performed using the same extract, if necessary, or separate extracts may be made for each test. Select and subdivide the sample according to the size indicated in Table 4. Remove particulate matter, such as lint and free particles, by treating each subdivided samples or negative control as follows. Place the sample into a clean 100-mL graduated hard glass cylinder with a glass-stopper, and add about 70 mL of water for injection. Shake for about 30 seconds, and drain the water. Repeat this step, and dry those pieces prepared for the extraction with vegetable oil in a dryer at a temperature not exceeding 50 ℃. [Note: Do not wipe the sample with a dry or wet cloth or rinse or wash with an organic solvent, surfactant, etc.]

Preparation of the extracts

Place a properly prepared sample in an extraction container, and add 20 mL of the appropriate extraction solvent. Repeat this procedure for each extraction solvent required for the test. Separately, prepare a 20-mL blank test solution of each medium for parallel injections and comparisons. Extract by heating in an autoclave at 121 ℃ for 60 minutes, in a thermostat at 70 ℃ for 24 hours, or at 50 ℃ for 72 hours. Allow sufficient time for the liquid in the container to reach the extraction temperature. [Note: The extraction conditions should not, under any circumstances, cause any physical changes such as melting or fusion of the sample pieces, resulting in a decrease in the available surface area. A slight adherence of the pieces is acceptable. Always add the washed pieces individually to the extraction medium. If culture tubes are used to extract vegetable oil in an autoclave, screw caps should be sealed adequately with pressure-sensitive tape.]

Cool to ordinary temperature but not below 20 ℃, shake vigorously for several minutes, and decant each extract immediately into a vessel dried under aseptic conditions. Store the extracts at a temperature of 20 to 30 ℃, and they should not be used for testing after 24 hours. Important matters include contact of the extraction medium with the available surface area used on the plastic, time and temperature during extraction, proper cooling, shaking, decanting, aseptic handling and storage of the extracts following extraction.

Table 4. Surface area of the sample to be used[1)]

Form of material	Thickness	Amount of the sample for each 20 mL of extraction solvent	Subdivided into
Film or sheet	< 0.5 mm	Equivalent of 120 cm^2 total surface area of both sides	Strips of about 5 × 0.3 cm
	0.5 - 1 mm	Equivalent of 60 cm^2 total surface area of both sides	

Slabs, tubing, and molded items	> 1 mm	Equivalent of 60 cm^2 total exposed surface area	Pieces less than about 5 × 0.3 cm
Rubber closures	> 1 mm	Equivalent of 25 cm^2 total exposed surface area	Do not subdivide[2)]

1) When surface area cannot be measured due to the structural form of the sample, use 0.1 g of a rubber closure per 1 mL of extraction solvent.
2) Molded rubber closures are tested as they are.

Method 1 Systemic injection test

This test is designed to evaluate systemic reactions to the extracts of test materials after injection into mice. When feasible, alternate injection routes may be used.

Test animals

Use healthy, not previously used albino mice weighing 17 - 23 g. In each test group, use only mice of the same species. Allow the animals to freely drink water and consume a diet for laboratory animals that is commonly used, with a known composition.

Procedure

Shake each extract vigorously before taking injection doses to ensure even distribution of the extracted matter. Inject the sample or the blank test solution as indicated in Table 5 to each of the five mice in the test group. However, the extract of the sample prepared with polyethylene glycol 400 and the corresponding blank test solution are diluted with 4.1 volumes of sodium chloride injection per g to obtain a solution with a concentration of about 200 mg of polyethylene glycol per mL.

Table 5. Injection procedure - Systemic injection test

Extract or blank test solution	Dose per kg	Route
Sodium chloride injection	50 mL	IV
A mixture of sodium chloride injection and ethanol (96) (19 : 1)	50 mL	IV
Polyethylene glycol 400	10 g	IP
Drug product vehicle (if applicable)	50 mL	IV
	50 mL	IP
Vegetable oil	50 mL	IP

IV: intravenous (aqueous sample and blank test solution);
IP: intraperitoneal (oleaginous sample and blank test solution).

Observe the animals immediately after injection, and 4 hours again after injection, then observe at least at 24, 48, and 72 hours. If none of the animals treated with the extract of the sample shows a significantly greater biological reactivity than the animals treated with the blank test solution during the observation period, the sample is considered to meet the requirements of this test. If two or more mice die, or if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a weightloss greater than 2 g occurs in three or more mice, the sample does not meet the requirements of the test. If any of the animals treated with the sample show only slight signs of biological reactivity, or if less than one animal shows overall biological reactivity or dies, repeat the test with 10 mice. In the repeated test, all 10 animals treated with the sample should show no significant biological reactivity over the animals treated with the blank test solution during the observation period.

Method 2 Intracutaneous test

This test is designed to evaluate local reactions to the extracts of test materials after injection into rabbits or guinea pigs.

Test animals

Select healthy, rabbits or guinea pigs whose fur can be shaved closely and has a skin free from mechanical irritation or trauma. When handling the animals, avoid touching the injection sites during observation periods, except to distinguish between edema and an oil residue.

Procedure

Shake each extract vigorously before taking injection doses to ensure even distribution of the extracted matter. On the day of the test, closely shave the fur on both sides of the animal's spine with forceps over a sufficiently large testing area. Avoid mechanical irritation and trauma. Remove disheveled hair under reduced pressure. If necessary, wipe the skin gently with diluted alcohol, and dry the skin prior to injection. If it is determined that the test results will not be affected, more than one type of extract from each rabbit or guinea pig may be used. Two animals are used for each sample, and the sample is injected intracutaneously, using one side of the animal for the sample and the other side for the blank test solution, as shown in Table 6. However, dilute each g of the extract of the sample prepared with polyethylene glycol 400, and the corresponding blank test solution with 7.4 volumes of sodium chloride injection to obtain a solution with a concentration of about 120 mg of polyethylene glycol per mL.

Table 6. Intracutaneous test

Extract or blank test solution	Number of sites per animal	Dose (μL) per site
Sample	5	200
Blank test solution	5	200

Examine the injection sites for evidence of any tissue reaction, such as erythema, edema, and necrosis. wipe the skin gently, if necessary, with diluted alcohol to facilitate reading of injection sites. Observe all animals at 24, 48, and 72 hours after injection. Rate the observations on a numerical scale for the sample extract and the blank test solution using Table 3. Clip the fur back as much as needed during the observation period. The average erythema and edema scores for the sample and blank sites are determined at scoring interval (24, 48, and 72 hours) per rabbit or guinea pig. After scoring at 72 hours, calculate the total score by adding all erythema scores and edema scores separately for each sample and blank test solution. Divide each total by 12 (2 animals x 3 scoring periods x 2 scoring categories) to determine the overall average score for each sample versus each corresponding blank test solution. The test requirements are met if the difference between the average score between the sample and blank test solution is 1.0 or less. If the average reaction to the sample is questionably greater than the average reaction to the blank test solution at any observation time, repeat the test using three additional rabbits or guinea pigs. The test requirements are met if the difference between the average score between the sample and blank test solution is 1.0 or less.

3) Functionality tests

These tests are performed on rubber closures used in injection needles. Resealability test is required only for closures intended for multiple-use containers.

The penetrability, fragmentation, and resealability of the rubber closure are tested by using a whole closure, and an air-dried rubber closure treated according to the test solution preparation of Eluate testing is used. For each test, a new needle with an outer diameter of 0.8 mm (21 gauge) and a lubricated long

bevel (bevel angle 12 ± 2°) is pierced perpendicularly to the surface of each closure without rotating.

A) Penetrability

Fill 10 suitable vials with water to the indicated volume, plug the closures with the sample, and secure with a cap. The required force for piercing is less than 10 N for each sample with an accuracy of ± 0.25 N.

B) Fragmentation

When using closures for liquid preparations, add water equivalent to the indicated volume minus 4 mL in 12 clean vials. Fit the rubber closures to be examined, secure with a cap, and allow to stand for 16 hours. When using rubber closures for dry preparations, fit rubber closures to be examined into 12 clean vials, and secure each with a cap. Using a hypodermic needle attached to a clean syringe, inject 1 mL into each vial of water while removing 1 mL of air. Repeat this procedure four times for each closure, piercing each time at a different site. Use a new needle for each closure, making sure that it is not blunted during the test. Filter the total volume of the liquid in all the vials through a single filter with a nominal pore diameter of 0.5 µm. When counting the rubber fragments, which are visible to the naked eye on the surface of the filter; it should be less than five. This limit is based on the assumption that fragments with a diameter exceeding 50 µm are visible to the naked eye. In case of doubt or controversy, the particles are examined microscopically to verify their nature and size.

C) Self-sealing capacity

Fill 10 vials with water,plug the vials with the sample, and secure them with the closure. Using a new hypodermic needle for each closure, pierce each closure 10 times at a different site. Immerse the vials in a 0.1% methylene blue solution, and reduce the external pressure by 27 kPa for 10 minutes. Restore to atmospheric pressure, and leave the vials immersed for 30 minutes. After rinsing the outside of the vials, there should be no blue tone vials.

Elemental Impurities
금속(원소)불순물시험법

The Elemental Impurities describes analytical procedures to measure the quantity of elemental impurities (including metalloid and non-metal elements) present in pharmaceuticals, using Inductively Coupled Plasma Spectrochemistry. When testing according to the Inductively Coupled Plasma Spectrochemistry method, if it has been demonstrated that the use of Inductively Coupled Plasma Spectrochemistry does not impact the test results, Atomic Absorption Spectroscopy (AAS) can be applied.

Unless otherwise specified, the acceptable value of elemental impurities in pharmaceuticals should not exceed the concentration limits outlined in the document "Evaluation and Control of Elemental Impurities in Drug Products". As the acceptable value may vary depending on the composition of the sample and the type of metal, there is a limit to presenting test solution preparation and manipulation methods for all samples. Therefore, in general, the analyst should verify and validate that the test procedure is appropriate for the instrument and sample used.

Sample preparation

Samples can be analyzed either directly or by dissolving it in an appropriate solvent, such as water or an organic solvent. When the sample is not soluble in water or organic solvents, decomposition techniques such as microwave-assisted decomposition method or similar ones can be employed. The sample prepared through the decomposition method should yield sufficient amount to allow quantification of each element at the limit specified in the corresponding monograph. The selection of the appropriate sample preparation depends on the nature of the sample and is the responsibility of the analyst. If it is necessary to add the metal to be analyzed at a certain concentration to the sample for proper signal intensity measurement, add a known amount of the same metal to the blank test solution, and if possible, use a solution that has the same amount as the sample. Standard solutions may contain multiple target elements. All reagents used for the preparation of the test and standard solutions should be free of elemental impurities, and all liquid samples should be weighed.

- **Microwave-assisted decomposition method**: It is a method that decomposes a sample using acid in a closed apparatus, which can minimize the loss of volatile elemental impurities. The choice of the acid depends on the sample matrix. The weights and volumes of the sample or solvent maybe adjusted according to the digestion apparatus used.
- **Standard solution**: A standard solution whose concentration has been confirmed, shall be diluted to a prescribed concentration using water or other solvents suitable for Inductively Coupled Plasma Spectrochemistry. The standard solution manufactured by a reference material producer (accredited by ISO guide 34) should be used. However, in the case of interference, it is desirable to match the properties of the standard solution to those of the test solution.

Test Method

Method 1 and 2 use Inductively Coupled Plasma-atomic Emission Spectrometry and Inductively Coupled Plasma-mass Spectrometry, respectively.

1) Method 1 (Inductively Coupled Plasma-atomic Emission Spectrometry)

Standard solution 1: Solution containing 1.5 *J* of the target element in a Matched matrix as the test solution.

Standard solution 2: Solution containing 0.5 *J* of the target element in a Matched matrix as the test solution.

Sample stock solution: Proceed as directed for the sample preparation above. (Allow the solution to cool, if necessary. For mercury determination, add an appropriate stabilizer.)

Test solution: Dilute the sample stock solution with an appropriate solvent to obtain a final concentration of the target elements at NMT 1.5 *J*.

Blank test solution: Matched matrix

Spectrometric system

Mode: ICP

Detector: Optical detection system

Rinse: Diluent used

Standardization: Standard solution 1, Standard solution 2, and Blank test solution

System suitability

Signal drift: Compare results obtained from Standard solution 1 before and after the analysis of the test solution.

Suitability criteria: Signal drift for each element is NMT 20%. (If samples are high in mineral content, rinse the system well before introducing the sample in order to minimize carryover)

Analysis: Analyze according to the manufacturer's analysis program and wavelength recommendations. Calculate and report results on the basis of the initial sample volume. Perform

appropriate corrections for interference effects due to the matrix (e.g., wavelength overlaps).

2) Method 2 (Inductively Coupled Plasma Mass Spectroscopy)

Standard solution 1: Solution containing 1.5 *J* of the target element in a Matched matrix.

Standard solution 2: Solution containing 0.5 *J* of the target element in a Matched matrix.

Sample stock solution: Proceed as directed for the sample preparation above (Allow the solution to cool, if necessary. For mercury determination, add an appropriate stabilizer).

Test solution: Dilute the Sample stock solution with an appropriate solvent to obtain a final concentration of the target elements at NMT 1.5 *J*.

Blank test solution: Matched matrix

Spectrometric system

Mode: ICP (An instrument with a cooled spray chamber is recommended. A collision cell or reaction cell may also be beneficial.)

Detector: Mass spectrometer

Rinse: Diluent used

Standardization: Standard solution 1, Standard solution 2, and Blank test solution

System suitability

Signal drift: Compare the results obtained from Standard solution 1 before and after the analysis of the test solution.

Suitability criteria: Signal drift for each element is NMT 20%. (If samples are high in mineral content, rinse the system well before introducing the sample in order to minimize carryover.)

Analysis: Analyze according to the manufacturer's analysis program and m/z recommendations. Calculate and report results on the basis of the initial sample volume. Perform appropriate corrections for interference effects due to the matrix (e.g., argon chloride interference with arsenic determinations).

Requirements for Test Method Validation

Limit test or quantitative test can be used depending on the purpose of the test method, and all the test methods should be verified to meet the validation requirements below.

1) Limit test

For limit tests, the suitability of the following validation parameters should be demonstrated using an appropriate test method validation procedure and a standard solution. The suitability of the method should be determined by adding a known quantity to each metal. At this time, the addition of the known amount is carried out before sample preparation steps are performed.

a) Detection sensitivity

Standard solution 1: Solution prepared at the accepted concentration limit for each element using reference materials.

Standard solution 2: Solution prepared at 80% of the accepted concentration limit for each element using reference materials.

Spiked test solution 1: Solution prepared after adding and pretreating a known amount of the sample with Standard solution 1.

Spiked test solution 2: Solution prepared after adding and pretreating a known amount of the sample with Standard solution 2.

Test solution: Solution prepared after pretreatment of the sample.

Acceptance criteria: The average value of the three repeated measurements of Spiked test solution 1 is within ±15% of the average value of the repeated measurements of the Standard solution 1. The average value of the replicate measurements of Spiked test solution 2 must provide a signal intensity or a value smaller than that of the Standard solution 1. The values obtained for each of the Spiked test solutions should be corrected using the result of the Test solution.

b) Precision

Test solutions: Six independent solutions prepared by spiking a known amount of the sample to Standard solution 1 of (A) Detection sensitivity

Acceptance criteria: NMT 20% relative standard deviation for each target element

c) Specificity

The test method must be able to clearly assess each element in the presence of components expected to be present in the sample (including other elements and matrix components).

2) Quantitative test

For quantitative tests, the suitability of the following validation parameters should be verified using an appropriate test method validation procedure and standard solution.

a) Accuracy

Standard solutions: Prepare solutions of at least 3 concentration levels ranging from 0.5 *J* to 1.5 *J*, including 0.5 *J*, of each element in a matched matrix using appropriate reference materials.

Test solutions: Add a pretreated known amount to the sample with at least 3 concentration levels ranging from 0.5 *J* to 1.5 *J*, including 0.5 *J*, of each element in the matched matrix using appropriate reference materials.

Acceptance criteria: 70%~150% for the mean recovery rate of three repeated measurements at each concentration

b) Precision

① Repeatability:

Test solutions: Six samples of independent reference materials (collected from the same lot) spiked with appropriate reference materials of the target element(s) at the acceptance limit.

Acceptance criteria: NMT 20% (N=6) relative standard deviation for each target element

② Laboratory precision

Perform the Repeatability analysis again either on a different day, with different test equipment, with a different analyst, or a combination of all. Combine the results of this analysis with those of the ① Repeatability analysis to make the total number of analyses 12.

Acceptance criteria: NMT 25% (N=12) relative standard deviation for each target element

c) Specificity

The test method must be able to clearly assess each element in the presence of components expected to be present in the sample (including other elements and matrix components).

d) Limit of Quantitation, Range, and Linearity

It is considered suitable if it meets the accuracy requirement in (A).

Terms

1) J

The concentration per diluted element considering the acceptance limit of each element, and the analysis range of the test equipment.

• For example, if the target elements are lead and arsenic for an analysis of an oral solid drug product with a daily dose of 10 g/day using ICP-MS, the acceptable limit value of these elements would be 0.5 μg/g and 1.5 μg/g, respectively. However, if the linear dynamic range of these elements ICP-MS is 0.01 ng/mL to 0.1 μg/mL, they must be diluted at least 100times to ensure that the analysis occurs in the linear dynamic range of the instrument. J value for the lead and arsenic would thus be equal to 5 ng/mL and 15 ng/mL, respectively, when the dilution factor is added.

Extractable Volume of Injections
주사제의 실용량시험법

The Extractable Volume of Injections is to ensure that the container is filled with a volume that can be taken slightly in excess of the labeled amount to be withdrawn Injections supplied in single-dose containers or multi-dose containers such as ampoules or plastic bags are usually filled with a sufficient volume of injection to administer the labeled amount, and the excess volume is determined depending on the characteristics of the product. Injectable suspensions and emulsions should be shaken and mixed before collecting the contents and measuring the density. If necessary, oily and viscous injections may be warmed according to the instructions on the label, and shaken immediately before transferring the contents. The contents should be cooled to 20 – 25 °C before measuring the volume.

Single-dose containers

Take 1 container if the labeled amount is 10 mL or more, 3 containers if the labeled amount is more than 3 mL and less than 10 mL, or 5 containers if the labeled amount is 3 mL or less, and collect the entire contents from each container. To collect the sample, use a dry syringe without exceeding 3 times the volume to be measured, fit with a 21-G needle of at least 25 mm in length. After expelling air bubbles from the syringe and needle, transfer the entire content of the syringe into a measuring cylinder and measure the volume, ensuring that the inside of the needle is not empty. Alternatively, the volume of the content (mL) can be calculated as the mass (g) divided by the density. As for the measuring cylinder, use a dry measuring cylinder with a measure capacity of 40% or more. In addition, when the labeled amount of the container is less than 2 mL, take an appropriate number of containers, use a separate dry syringe for each container to collect the entire content, and measure the entire volume. If the labeled amount of the container is 10 mL or more, it is allowed to open the container, and the entire content can be measured by directly placing it into a measuring cylinder or a tared beaker with a known volume.

The volume of each container is greater than the labeled amount. If the labeled amount is 2 mL or less, the sum of the combined content is measured, and the volume of the combined content is greater than the sum of the labeled amounts.

Multi-dose containers

For multi-dose injections with the single dose and number of administrations indicated, take 1 container and proceed as directed for single-dose containers using the same number of separate dry syringe units as the specified number of administrations.

The volume from each syringe is greater than the indicated single dose.

Cartridges and prefilled syringes

Take 1 container if the labeled amount is 10 mL or more, 3 containers if the labeled amount is 3 mL or more and less than 10 mL, or 5 containers if the labeled amount is 3 mL or less. If the accessories including needles, plungers, syringes, etc. are available attach them to the container, and after making sure that the needle is not empty, transfer the entire content of each container into a dry tared beaker by slowly and constantly pressing the plunger. Calculate the volume (mL) by dividing the mass (g) by the density.

The volume from each preparation is greater than the labeled amount.

Parenteral infusions

Take 1 container and transfer the entire content into a dry measuring cylinder with a measuring capacity of 40% or more. Measure the volume transferred.

The volume is greater than the labeled amount.

Fats and Fatty Oils
유지시험법

The Fats and Fatty Oils is applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and other similar substances.

Preparation of the sample

If the sample is in a solid form, melt it carefully and filter it with a dry filter paper if necessary. If the sample is turbid, heat it to approximately 50 °C. If the sample remains turbid, filter it with a dry filter paper while it is still warm. In all cases, ensure that the sample is thoroughly mixed to achieve homogeneity.

Melting point

Proceed by Method 2 described in the Melting Point.

Congealing temperature of fatty acids

1) Preparation of fatty acids

Dissolve 75 g of potassium hydroxide dissolved in 100 g of glycerin, add 75 g of the solution to a 1000-mL beaker, and heat it to 150 °C. Add 50 g of sample to this mixture, stir occasionally, heat for about 15 minutes, and saponify completely. During this process, ensure that the temperature does not exceed 150 °C. Cool the solution to 100 °C, dissolve by adding 500 mL of hot water, and slowly add 50 mL of diluted sulfuric acid (1 in 4). Heat the solution while stirring occasionally until the clear layer of fatty acid is clearly separated. Separate the fatty acid layer and wash it several times with hot water until the washed solution no longer shows acidity with methyl orange TS. Then, transfer the resulting solution to a small beaker. Heat it on a water bath until the fatty acid layer becomes clear due to water separation. Filter the hot fatty acid solution, transfer the resulting solution to a small beaker, and heat it carefully to 130 °C to remove water.

2) Measurement of congealing temperature

Proceed by the method described in the Congealing Temperature.

Specific gravity

1) Liquid sample at ordinary temperature

Proceed by the method described in the Determination of Specific Gravity and Density.

2) Solid sample at ordinary temperature

Unless otherwise specified, fill a pycnometer with water at 20 °C and weigh it accurately. After discarding the water and drying, accurately weigh the pycnometer. Then, fill the melted sample to approximately 3/4 of the depth of the pycnometer, and allow to stand for 1 hour at a temperature slightly higher than the melting temperature of the sample to completely remove any air remaining in the sample. After adjusting to the specified temperature, weigh the pycnometer accurately. Fill the sample with water at 20 °C, and weigh accurately again. Other procedures should follow the Specific gravity and density described in Method 1.

$$d = \frac{M_1 - M}{(M_2 - M) - (M_3 - M_1)}$$

M: Mass of the pycnometer (g)

M1: Mass of the pycnometer filled with the sample (g)

M2: Mass of the pycnometer filled up with water (g)

M3: Mass of the pycnometer filled up with the sample and water (g)

Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize 1 g of the sample.

Procedure Unless otherwise specified, accurately weigh the amount of sample specified in Table 1 according to the acid value of the sample, place it in a 250-mL flask with a stopper, add 100 mL of a mixture of ether and ethanol (95) (1:1 or 2:1), and dissolve the sample by heating, if necessary. Then, add a few drops of phenolphthalein TS, and titrate with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color that persists for 30 seconds. If the solution turns turbid when cooled down, titrate when it's hot. Before using this solvent, use as phenolphthalein TS as an indicator and add 0.1 mol/L potassium hydroxide-ethanol VS until it develops a light red color for 30 seconds.

$$\text{Acid value} = \frac{\text{0.1 mol/L potassium hydroxide} \cdot \text{ethanol consumption (mL) X 5.611}}{\text{amount (g) of sample}}$$

Table 1

Acid value	Amount (g) of sample collected
NMT 5	20
NLT 5 and less than 15	10
NLT 15 and less than 30	5
NLT 30 and less than 100	2.5
NLT 100	1.0

Saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the ester and neutralize the free acid in 1 g of the sample.

Procedure Unless otherwise specified, accurately weigh 1 to 2 g of the sample, transfer it to a 200-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a small reflux condenser or air condenser (length: 750 mm, diameter: 6 mm) to the flask, and heat gently on a water bath for 1 hour with occasional shaking. Cool down the solution, add 1 mL of phenolphthalein TS, and titrate the excess potassium hydroxide immediately with 0.5 mol/L hydrochloric acid VS. If the test solution turns turbid when cooled down, titrate it when it's hot. Perform a blank test with the same method.

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{Amount (g) of sample}}$$

a: Volume of 0.5 mol/L hydrochloric acid VS consumed in the blank test solution (mL)

b: Volume of 0.5 mol/L hydrochloric acid VS consumed in titration of the test solution (mL)

Ester value

The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the ester in 1 g of the sample.

Procedure Unless otherwise specified, the saponification value and the acid value are measured and the resulting difference is taken as the ester value.

Hydroxyl value

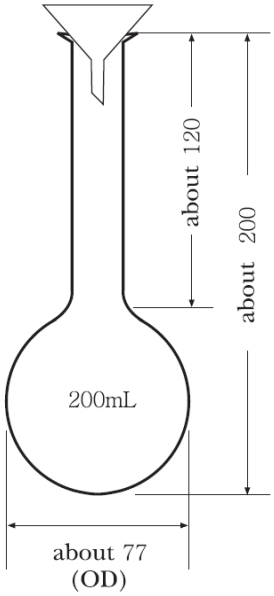
The hydroxyl value is the number of milligrams of potassium hydroxide (KOH) required to neutralize acetic acid combined with hydroxyl groups, when 1 g of the sample is acetylated by the following procedure.

Procedure Accurately weigh about 1 g of the sample, place it in a 200-mL round-bottom flask (figure), and add exactly 5 mL of acetic anhydride·pyridine TS. Place a small funnel on the neck of the flask, heat it by immersing it up to about 1 cm from the bottom in an oil bath maintained at a between 95 °C and 100 °C. To protect the neck from the oil bath heat, place a thick, round paper with a round hole on the joint of the flask under the neck. After 1 hour, remove the flask from the oil bath, and cool it down. Add 1 mL of water through a funnel, and shake to decompose the acetic anhydride. Again, heat the flask in the oil bath for 10 minutes. After cooling, wash the funnel and the flask neck with 5 mL of neutralized ethanol, and titrate with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL of phenolphthalein TS). Perform a blank test using the same method.

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{Amount (g) of sample}} + \text{Acid value}$$

a: Volume of 0.5 mol/L potassium hydroxide ethanol VS consumed in the blank test solution (mL)

b: Volume of 0.5 mol/L potassium hydroxide ethanol VS consumed in titration of the test solution (mL)



*OD: Outer diameter
*The figures are in mm.

Figure

Unsaponifiable matter

The amount of unsaponifiable matter is calculated by subtracting the amount of fatty acids calculated from the amount of oleic acid, from the amount of substance that is unsaponifiable following the procedure described below. It is soluble in ether, but insoluble in water. The limit is expressed in percentage (%) in the monograph.

Procedure Weigh accurately about 5 g of the sample, and place it in a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS. Attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with occasional shaking, and transfer it to the first separatory funnel. Wash the flask with 100 mL of warm water, and transfer the washed solution to the first separatory funnel. Further, add 50 mL of water to the separatory funnel, and cool the resulting solution to ordinary temperature. Wash the flask with 100 mL of ether, transfer the washed solution to the first separatory funnel, perform extraction by vigorously shaking it for 1 minute, and let the solution stand until both layers are clearly separated. Put the water layer into the second separatory funnel, add 50 mL of ether, mix by shaking and allow to stand in the same way. Transfer the water layer to the third separatory funnel, add 50 mL of ether, and perform extraction by shaking again in the same way. Transfer the ether extracts of the second and third separatory funnels into the first separatory funnel, wash each separatory funnel with a small amount of ether, and combine the washed solution into the first separatory funnel, Wash the first separatory funnel with 30 mL of water, until the washed solution no longer shows a light red color with 2 drops of phenolphthalein TS. Add a small amount of anhydrous sodium sulfate to the ether extracts, and allow to stand for 1 hour. Filter the ether extracts with a dry filter paper, and collect the filtrates into a pre-weighed flask. Wash the first separatory funnel well with ether, filter the washed solution using the previously-used filter paper, and add it to the flask. After almost evaporating the filtered and washed solution completely on a water bath, add 3 mL of acetone, and evaporate to dryness in the water bath. After drying it for 30 minutes at a temperature between 70 °C and 80 °C under reduced pressure (about 2.67 kPa), transfer it to a desiccator (reduced pressure, silica gel), let it cool for 30 minutes, and weigh the mass accurately. Add 2 mL of ether and 10 mL of neutralized ethanol, and dissolve the extracts by shaking well. Then, add a few drops of phenolphthalein TS, and titrate the remaining fatty acids in the residue with 0.1 mol/L potassium hydroxide-ethanol VS until a light red color that persists for 30 seconds appear.
Amount (g) of sample

$$\text{Unsafonifable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{Amount (g) of sample}} \times 100$$

a: Amount of the extracts (g)
b: Volume of 0.1 mol/L potassium hydroxide ethanol VS consumed (mL)

Iodine value

The iodine value is the number of grams (g) of halogen converted to iodine (I) that combines with 100 g of the sample under the following conditions.

Procedure Unless otherwise specified, weigh accurately the amount of sample shown in Table 2 using a small glass container, according to the expected iodine value of the sample. Place the container containing the sample in a 500-mL glass-stoppered flask, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL of Wijs' TS, and mix well. Keep tightly closed, shield from light,, and allow to stand at 20 °C to 30 °C for 30 minutes (1 hour when the expected iodine value is more than 100) with occasional shaking. Add 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and mix by shaking. Then, titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank test using the same method.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{Amount (g) of sample}}$$

a: Volume of 0.1 mol/ L sodium thiosulfate VS consumed in the blank test solution (mL)
b: Volume of 0.1 mol/L sodium thiosulfate VS consumed in titration of the test solution (mL)

Table 2

Iodine value	Amount (g) of sample collected
NMT 30	1.0
NLT 30 and NMT 50	0.6
NLT 50 and NMT 100	0.3
NLT 100	0.2

Flame Coloration
불꽃반응시험법

The Flame Coloration is a method to identify certain type of elements by their characteristic to produce their own color in a colorless flame of a sensitive Bunsen burner.

1) Metal salt

The platinum wire used for this test is about 0.8 mm in diameter and straight at the end.

In the case of a solid sample, make it into a slurry by adding a small amount of hydrochloric acid. Apply the small amount of the slurries up to about 5-mm from the tip of the platinum wire

and perform the test by holding it horizontally and placing it in a colorless flame. In the case of a liquid sample, immerse the tip of the platinum by about 5 mm into the sample. Carefully lift it from the sample and perform the test in the same way as for the solid sample.

2) Halides

Cut a copper mesh, which was made of copper wires with a diameter of 0.174 mm and a gap of 0.25 mm, into pieces with a width of approximately 1.5 cm and a length of about 5 cm, and wrap them around one end of the copper wire. Ignite it in the colorless flame of the Bunsen burner until the flame no longer appears green or blue, and then let it cool. Repeat this operation several times to completely coat the copper oxide. After cooling, apply 1 mg of the sample onto the copper mesh, ignite and burn it unless otherwise specified. Repeat this operation 3 times and then perform the test by placing the copper mesh in the colorless flame.

The description “flame coloration persists” means that the reaction persists for about 4 seconds.

Fluorescence Spectroscopy
형광광도법

The Fluorescence Spectroscopy measures the intensity of fluorescence emitted from a solution containing a fluorescent substance when irradiated with excitation light within a specific wavelength range. The technique also applies to phosphorescent substances.

In a dilute solution, the fluorescence intensity F is proportional to the concentration c and the layer length l of the fluorescent substance in the solution.

$$F = k \cdot P_0 \cdot \phi \cdot \varepsilon \cdot c \cdot l$$

k: Constant,
P_0: Intensity of excitation light,
ϕ: Quantum yield of fluorescence or phosphorescence.

$$\text{Quantum yield } \phi = \frac{\text{Number of fluorescence or phosphorescence quanta emitted}}{\text{Number of excitation quanta absorbed}}$$

ε: Molar extinction coefficient of the substance at the excitation light wavelength

Apparatus

In general, a spectrofluorometer is employed. Light sources that ensure stable excitation light, such as xenon lamps, lasers, or alkaline halide lamps are used. A non-fluorescent quartz cell (1 cm × 1 cm) with four transparent sides is usually used as the test solution container.

Procedure

The excitation spectrum is obtained by measuring fluorescence intensity of the test solution across varying excitation wavelengths with a constant emission wavelength, and drawing a curve showing the relationship between the excitation wavelength and fluorescence intensity. The fluorescence spectrum is obtained by monitoring the fluorescence intensity of the diluted test solution while fixing the excitation light at an appropriate wavelength while changing the fluorescence wavelength little by little to obtain a curve showing the relationship between the emission wavelength and the fluorescence intensity. If required, correct the spectrum considering the optical properties of the apparatus.

The fluorescence intensity is usually gauged near the maximum wavelength of the excitation and fluorescence spectrum of the fluorescent substance. Due to its sensitivity to slight changes in measurement conditions, the fluorescence intensity of a standard solution is measured and compared. Unless otherwise specified, perform the following operations using the standard solution, test solution, and control solution prepared as prescribed in the monograph. Fix the excitation and fluorescence wavelength scales as designated, adjust the emission of blank to zero, insert a quartz cell with the standard solution into the light path, and adjust the instrument so that the standard solution displays a fluorescence intensity between 60% and 80% of the full scale. Under the same condition, measure the fluorescence intensity (%) of the test solution and control solution. Adjust the wavelength width as necessary unless otherwise specified.

Note

Factors like concentration, temperature, and pH of the solution, along with the nature and purity of the solvent or reagents used, can influence fluorescence intensity.

Foreign Metallic Matter
금속성이물시험법

The Foreign Metallic Matter is a method to test the existence of foreign metallic matters in the ophthalmic ointments.

Preparation of sample

Take 10 samples of ophthalmic preparations, weigh 5 g of each sample in a clean environment, and place them individually in a flat-bottomed Petri dish with a diameter of 60 mm. Cover and heat at 85 to 110 ℃ for 2 hours to dissolve the sample completely. Let it harden at ordinary temperature being careful not to shake it. If the sample volume is less than 5 g, take out the entire content as much as possible, and operate as described above.

Procedure

Invert each Petri dish and observe with a micrometer-equipped microscope with at least 40 times magnification. Direct an illuminator from above the sample at a 45° angle and count the total number of the metallic particles that are larger than 50 μm on the bottom of each Petri dish.

Use Petri dishes with a clean bottom and free from foams and scratches, and if possible, the angle of the inner circumference and bottom should be as perpendicular as possible.

Evaluation

The sample is considered to be suitable if the total number of metallic particles counted in all 10 samples does not exceed 50, and if less than 1 Petri dish is found to contain more than 8 metallic particles. When these requirements are not met, repeat the above procedures on 20 additional samples. When testing 30 samples, it is considered to be suitable if the total number of metallic particles larger than 50 μm does not exceed 150 in all 30 samples tested, and if less than 3 Petri dishes are found to contain more than 8 metal particles, respectively.

Gamma-ray Spectrometry
감마선 측정법

The Gamma-ray Spectrometry is a method of measuring gamma-rays and X-rays in radiation emitted by radionuclides.

Assay by Gamma-ray spectrometry is performed according to the assay by gamma-ray spectrometer, scintillation counter, or ionization chamber, unless otherwise specified. The gamma-ray spectrometer is used for the identification of nuclides, detection of different nuclides and assay of them. The scintillation counter or ionization chamber is used for assay of unknown different nuclides where the amount of them is less than that specified in the monograph due to the limited nuclides.

1) Spectroscopy by gamma-ray spectrometer

The identification of nuclides and the detection of different nuclides by this method are performed by measuring the spectrum of gamma-rays emitted from the sample using a gamma-ray spectrometer, or, if necessary, measuring the gamma-ray spectrum again under the same conditions after a certain period of time, and comparing them.

Procedure Unless otherwise specified, proceed by the following method.

Measure the gamma-ray spectrum of the gamma-ray standard source, of which nuclide has been confirmed in advance, by placing it at a certain distance from the gamma-ray detector. Determine the relationship between the spectral peak (hereinafter referred to as "peak") and the gamma-ray energy due to the photoelectric effect at appropriate intervals from low to high energy to create an energy calibration curve of the spectrometer.

Measure the gamma-ray spectrum of an appropriate amount of sample, and determine its nuclide by obtaining the peak energy appeared in the spectrum using the energy calibration curve. If it is difficult to identify the nuclide only with the obtained gamma-ray spectrum, measure the gamma-ray spectrum of the same sample again under the same conditions after a certain period of time. Then, determine the nuclide by its energy and half-life from the change ratio over time of the peak energy of interest and the count rate of peak area in the gamma-ray spectrum.

2) Assay by gamma-ray spectrometer

Assay by this method is performed by calculating the count rates of the representative gamma-ray peak areas emitted from the sample and reference standard.

Procedure Unless otherwise specified, proceed by the following method.

A) In case of using a reference standard with the same nuclide as the sample

Weigh accurately a certain amount of the sample and reference standard, add a solvent to each of them, if necessary, and dilute them to make the test and standard solutions. Put the same amount of the test and standard solutions separately into measuring container made of the same material and shape, and measure their gamma-ray spectra at a certain distance from the gamma-ray detector. Measure the gamma-ray spectrum according to the spectroscopic method by gamma-ray spectrometer and calculate the count rate of spectrum peak area to determine the radioactivity in a certain amount of sample according to the following equation:

Radioactivity in a certain amount of sample

$$= S \times \frac{A}{A'} \times \frac{D}{D'} \times G$$

S: Radioactivity in a certain amount of reference standard
A: Count rate of the same peak area of the test solution
A': Count rate of the same peak area of the standard solution
D: Dilution factor of the test solution
D': Dilution factor of the standard solution
G: Correction terms of geometric conditions, such as the position of test and standard solutions

However, use G = 1 as much as possible.

B) In case of using a reference standard with the different nuclide from the sample

Measure the gamma-ray spectrum of the gamma-ray standard source, of which radioactivity has been accurately quantified, according to method by gamma-ray spectrometer. Create the peak count efficiency curve by calculating the detection efficiency of the peak area in an appropriate gamma-ray energy range, considering the shape of the standard source and the distance to the gamma-ray detector, etc. Accurately weigh a certain amount of the sample, dilute it with a solvent, if necessary, and use it as the test solution. Put the test solution in an appropriate measuring container and measure the gamma-ray spectrum under the same conditions as the standard source. Calculate the count rate of the peak area of the gamma-ray of interest in the measured spectrum and obtain the radioactivity in a certain amount of the sample according to the following equation:

Radioactivity in a certain amount of sample

$$= \frac{N_X}{F_X} \times \frac{1}{R} \times D \times R$$

N_X: Count rate of the peak area of the test solution
F_X: Energy detection efficiency obtained from the peak count efficiency curve
R: Gamma-ray emission rate of peak energy
D: Dilution factor of the test solution
G: Correction terms of geometric conditions, such as the position of test solution and standard source

However, use G = 1 as much as possible.

Also, determine the radioactivity of different nuclide in the same way.

3) Assay by scintillation counter

Assay by this method is performed by counting the gamma-rays emitted from the sample and reference standard under the same conditions using a scintillation counter, and comparing them.

Procedure Unless otherwise specified, use the reference standard with same nuclide as the sample and proceed by the following method.

Weigh accurately a certain amount of the sample and the reference standard, add a solvent to each of them, if necessary, and dilute them to make the test and standard solutions. Put the same amount of the test and standard solutions separately into each measuring container made of the same material and shape. Measure each radioactivity under the same conditions using a scintillation counter and calculate the radioactivity in a certain amount of sample according to the following equation:

Radioactivity in a certain amount of the sample

$$= S \times \frac{A}{A'} \times \frac{D}{D'} \times G$$

S: Radioactivity in a certain amount of reference standard
A: Count rate of the test solution
A': Count rate of the standard solution
D: Dilution factor of the test solution
D': Dilution factor of the standard solution
G: Correction terms of geometric conditions, such as the position of test and standard solutions, etc.

However, use G = 1 as much as possible.

4) Assay by ionization chamber

Assay by this method is performed by measuring the ionization current or the converted indication value (hereinafter referred to as "ionization current") for the gamma-rays emitted from the sample and reference standard under the same conditions using an ionization chamber, and comparing them.

Procedure Unless otherwise specified, proceed by the following method.

A) In case of using a reference standard with the same nuclide as the sample

Weigh accurately a certain amount of the sample and reference standard of which radioactivity is known, add a solvent to each of them, if necessary, and dilute them to make the test and standard solutions. Put the same amount of test and standard solutions separately into each measuring container made of the same material and shape. Place the containers at a certain position in an ionization chamber and measure each ionization current to calculate the radioactivity in a certain amount of sample according to the following equation:

Radioactivity in a certain amount of sample

$$= S \times \frac{I}{I'} \times \frac{D}{D'} \times G$$

S: Radioactivity in a certain amount of reference standard
I: Ionization current of the test solution
I': Ionization current of the standard solution
D: Dilution factor of the test solution
D': Dilution factor of the standard solution
G: Correction terms of geometric conditions, such as the position of test and standard solutions.

However, use G = 1 as much as possible.

B) In case of using a reference standard with the different nuclide from the sample

If it is difficult to obtain the same nuclide as the sample, use a reference standard of which radioactivity is accurately identified in advance with an ionization chamber calibrated according to the correct method. Determine the relationship between the ionization current and the radioactivity, measure that of the sample under the same conditions, and compare them.

Weigh accurately a certain amount of the sample, dilute it with a solvent if necessary, and use it as the test solution. Measure the ionization current under the same conditions as when measuring the reference standard in the ionization chamber, and determine the radioactivity in a certain amount of sample according to the following equation:

Radioactivity in a certain amount of the sample

$$= I \times K \times D \times G$$

I: Ionization current of the test solution
K: Integer converting ionization current to radioactivity
D: Dilution factor of the test solution
G: Correction terms of geometric conditions, such as the position of test and standard solutions.

However, use G = 1 as much as possible.

C) In the case of using a reference standard with the different nuclide from the sample, which contains two types of nuclides

If it is difficult to obtain the same nuclide as the sample, use a reference standard of which radioactivity is accurately identified in advance with an ionization chamber calibrated according to the correct method. Determine the relationship between the ionization current and the radioactivity when measured using a lead container with a specific thickness (1 to 5 mm) and when measured without using it, and determine the radioactivities of two nuclides by measuring that of the sample under the same conditions, and comparing them.

Weigh accurately a certain amount of sample, dilute it with the same solvent used in the standard solution, if necessary, and use it as the test solution. Put the same amount of test solution as the standard solution used for calibration into a measuring container made of the same material and shape. Measure the ionization current in an ionization chamber under the same conditions as measuring for the reference standard and calculate the radioactivity in a certain amount of sample according to the following equation:

Radioactivity of nuclide A in a certain amount of sample

$$= \frac{\beta I - I'}{\beta - \alpha} \times K_A \times D$$

Radioactivity of nuclide B in a certain amount of sample

$$= \frac{I' - \alpha I}{\beta - \alpha} \times K_B \times D$$

I: Ionization current measured without using a lead container
I': Ionization current measured using a lead container
K_A: Integer converting ionization current of nuclide A to radioactivity
K_B: Integer converting ionization current of nuclide B to radioactivity
α: Decay rate of the ionization current of nuclide A using a lead container
β: Decay rate of the ionization current of nuclide B using a lead container
D: Dilution factor of the test solution

Gas Chromatography
기체크로마토그래프법

The Gas Chromatography is a method in which a sample mixture is injected into a column coated with a suitable stationary phase, and an inert gas (carrier gas) is used as the mobile phase to separate and analyze each component by using the difference in retention for the stationary phase. This method can be applied to gaseous or vaporizable samples and is used for purposes such as identification, purity test, and assay. Each component of the

mixture injected into the column is distributed in the mobile and stationary phases in a unique ratio, k.

$$k = \frac{\text{Amount of compound in the stationary phase}}{\text{Amount of compound in the mobile phase}}$$

Since there is the following relationship between this ratio *k*, the column passing time t_0 (time from the injection of a substance with $k = 0$ to the peak maximum of that substance), and the retention time t_R (time from the injection of the analyte to the peak maximum of that analyte) of the mobile phase, the retention time becomes a unique value of the substance under the same conditions.

$$t_R = (1 + k)t_0$$

Apparatus

Usually, the apparatus consists of a carrier gas, an injection port, a flow controller, a column, a column oven, a detector, and a recorder. If necessary, devices for introducing combustion gas, burning supporting gas, and auxiliary gas, flow controller and sample injection device for headspace, etc. can be used. The carrier gas introducing injection port and flow controller deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure control valve, a flow control valve, a pressure gauge, etc. The sample injection port is used to introduce a certain amount of sample into the carrier gas channel with high accuracy and reproducibility, and is available for packed columns and capillary columns. For the sample injection port for capillary columns, there are devices that use the split injection method and those that use the split-less injection method. There are usually two types of columns: packed columns and capillary columns. Packed columns are tubes made of inert metal, glass, or synthetic resin filled uniformly with a packing material for gas chromatography of a certain size. Packed columns with an internal diameter of NMT 1 mm are also referred to as packed capillary columns (micro-packed columns). Capillary columns are tubes with an empty center and a stationary phase for gas chromatography on the inner surface of an inert metal, glass, quartz or synthetic resin tube. The column oven has a temperature controller to maintain a constant column temperature and has a volume that can accommodate a column of the desired length. The detector is used to detect components separated from the column, and types can include alkaline thermal ionization detector, flame photometric detector, mass spectrometer, flame ionization detector, electron capture detector, thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

Procedure

Unless otherwise specified, proceed through the following method. After setting up the apparatus in advance, use the detector, column, and carrier gas under the operating conditions specified in the monograph to flow the carrier gas at the specified flow rate, and equilibrate the column to the specified temperature. Inject the test solution or standard solution in the amount specified in the monograph through GC inlet system. The separated components are detected by the detector and a chromatogram is obtained on the recorder.

Identification and purity

In identification, check whether the retention times of the analyte in the test solution and the standard analyte are the same or whether the peak shape of the analyte does not change even when the standard analyte is added to the test solution. Purity test is usually performed using a standard solution at a concentration corresponding to the limit of the impurity in the test solution or by the percentage peak area method. Unless otherwise specified, the isomer ratio of the test solution is determined using the percentage peak area method. In the percentage peak area method, the sum of the peak areas of individual peaks obtained from the chromatogram is set to 100, and the composition ratio is determined from the peak area ratio of each component. However, to obtain an accurate composition ratio, the peak area should be corrected using the response factor to the principal component of the mixture.

Assay

Usually the internal standard method is followed. However, the absolute calibration curve method is used when a suitable internal standard is not available. The standard addition method is used when the impact of components other than the analyte on the quantitative determination result is not negligible against a result of the determination.

1) Internal standard method

In the internal standard method, a stable compound should be chosen as the internal standard, with retention time as close as possible to that of the analyte while ensuring complete separation from all other peaks of the sample. Prepare several standard solutions with concentrations adjusted stepwise by adding the reference standard of the analyte to a certain amount of the internal standard specified in the monograph. From the chromatogram obtained by injecting a certain amount of these standard solutions, determine the ratio of the peak area or peak height of the standard analyte to that of the internal standard. Plot a calibration curve with this ratio on the vertical axis and the amount of standard analyte or the ratio of the amount of the standard analyte to the amount of internal standard on the horizontal axis. This calibration curve is usually a straight line passing through the origin. Next, obtain a chromatogram under the same analytical conditions as when plotting a calibration curve by preparing a test solution with the same amount of the internal standard according to the method specified in the monograph. Determine the ratio of the peak area or peak height of analyte to that of the internal standard and calculate the amount of analyte from the calibration curve. In the monograph, a standard solution that falls within the concentration range where the calibration curve is a straight line and a test solution with a concentration close to the concentration of the standard solution are usually prepared. After injecting each amount of those solutions specified in the monograph, perform a test using the gas chromatography method to calculate the amount of the analyte under the same analytical conditions.

2) Absolute calibration curve method

Prepare several standard solutions of standard analyte with stepwise concentrations and inject a certain amount of each standard solution accurately and reproducibly to obtain a chromatogram. From the chromatogram obtained, prepare a calibration curve by putting the peak area or peak height of the standard analyte on the vertical axis and the amount of the standard analyte on the horizontal axis. This calibration curve is usually a straight line passing through the origin. Next, prepare a test solution according to the method specified in the monograph, obtain a chromatogram under the same analytical conditions as when preparing a calibration curve, measure the peak area or peak height of the analyte, and determine the amount of the analyte from the calibration curve. In the monograph, a standard solution that falls within the concentration range where the calibration curve is a straight line and a test solution with a concentration close to the concentration of the standard solution are usually prepared. After

injecting each amount of those solutions specified in the monograph, perform a test using the gas chromatography method to obtain the amount of the analyte under the same analytical conditions. In this method, all procedures must be carried out under strictly constant conditions.

3) Standard addition method

Take exactly 4 or more test solutions in a certain amount. Accurately add the standard solution of the analyte to these test solutions, except for one, to give them stepwise concentrations. Dilute these solutions and the previously excluded solution to a certain amount and use them as the test solutions. Calculate each peak area or peak height from the chromatogram obtained by injecting a specific amount of these solutions accurately and reproducibly. Calculate the increase of analyte through the addition of standard solution from the concentration of analyte put into each test solution. Plot a correlation line by positioning the above increments on the horizontal axis and peak area or peak height on the vertical axis. Obtain the amount of analyte from the distance between the intersection of the horizontal axis of the correlation line and the origin. This method can also be applied when the calibration curve of the analyte prepared by the absolute calibration curve method is a straight line passing through the origin. Also, all measurement procedures must be carried out under strictly constant conditions.

Method for peak measurement

Generally, the following methods are used.

1) Peak height measuring method

A) Peak height method

Measure the length from the intersection point where the vertical line drawn from the apex of the peak to the horizontal axis of the recording paper meets the tangent (baseline) connecting both ends of the peak to the peak's apex.

B) Automatic peak height method

Measure the peak height of the signal from the detector with a data processing device.

2) Peak area measuring method

A) Width at half-height method

Multiply the peak width at the half-height by the peak height.

B) Automatic integration method

Measure the peak area of signal from the detector using the data processing device.

System suitability

System suitability is an essential item in a test method that uses a chromatographic procedure, and is performed whenever a test is performed using the system to check whether the system used for drug testing has adequate performance. The test procedures and acceptance criteria of system suitability must be prescribed in the test method of drug quality standards. If the prescribed acceptance criteria are not met, the results of drug quality tests using the system cannot be considered acceptable.

System suitability is evaluated in terms of "system performance" and "system repeatability", and in the case of a purity test, the term "test for required detectability" may also be evaluated.

1) Test for required detectability

By confirming that the peak of the target related substance is reliably detected at the concentration of the standard limit in a purity test, it is verified that the system in use has the performance required to achieve the purpose of the test.

In a quantification test under the Purity, the "Test for required detectability" section is usually required to define the degree of the response when the solution is injected at the standard limit, which indicates that the response has linearity around the limit. It is not required to set a "Test for required detectability" section in the following cases: when a standard solution with the same concentration as the limit is used to compare the degree of response of the test solution in tests such as a limit test; or, when the detection at the limit level can be confirmed by the "system repeatability," etc.

2) System performance

By confirming that specificity for the sample is assured, it is verified that the system in use has the necessary performance to achieve the purpose of the test.

In assay, "System performance" should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting substance is preferable) and, if appropriate, by the order of their elution. In purity test, both the resolution and the order of elution should be specified between the target substance and a substance to be separated (a closely eluting substance is preferable). In addition, the symmetry factor should be specified along with the sample, if necessary. However, if there is no suitable target substance to be separated, the "system performance" can be defined based on the number of theoretical plates and the symmetry factor of the test ingredient.

3) System repeatability

By confirming that the degree of variation (precision) of the response of the test ingredient is adequate for the purpose of the test when the standard or system suitability solution is repeatedly injected, it is verified that the system has the performance required to achieve the purpose of the test.

The tolerance of system repeatability is usually defined as the relative standard deviation (RSD) of the response of the target component obtained through the repeated injections. The system repeatability can also be confirmed by repeating the injection of the standard solution before the injection of the test solution, by dividing the standard solution before and after the injection of the test solution, and by injecting the standard solution between injections of test solution.

In principle, the total number of repeated injections should be 6. However, when a long time is required for one-time analysis, such as when analyzing by the gradient elution or when analyzing samples containing late eluting components, it may be acceptable to decrease the number of repeated injections by establishing a new "system repeatability" tolerance that guarantees a level of "system repeatability" equivalent to that at 6 repeated injections.

The tolerance of system repeatability is set at an appropriate level, considering the data reviewed for the application of the test method, the precision required for the test, etc.

Point to consider on changing the operation conditions

Among the operating conditions specified in the monograph, the following may be partially modified within the scope suitable for system suitability regulation: internal diameter and length of the column, particle size of the packing material, concentration or thickness of the stationary phase, column temperature, heating rate, type and flow rate of the carrier gas, and split ratio. However, the sample injection port for headspace and its operating conditions can be modified within the scope that allows for accuracy and precision beyond that of a specified method.

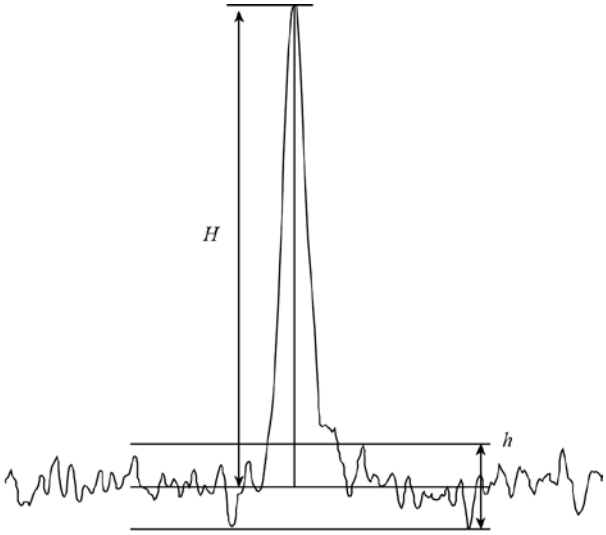
Terminology

S/N ratio (Signal to Noise ratio):
Defined by the following equation:

$$\mathrm{S/N} = \frac{2H}{h}$$

H: Peak height from the baseline of the target component peak (the median value of background noise)
h: Width of background noise of the chromatogram of test solution or blank test solution around the peak of the target component

The baseline and background noise are measured over a range 20 times the peak width at the half of the peak height of the target component. When using a blank test solution, measure over nearly the same range as described above around the point where the target component is measured.



Symmetry factor
The symmetry factor indicates the degree of symmetry of a peak in the chromatogram and is defined as S in the following equation:

$$S = \frac{\mathrm{W_{0.05h}}}{2f}$$

$W_{0.05h}$: Width of the peak at one-twentieth of the peak height from the baseline.
f: Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height,

where $W_{0.05h}$ and *f* have the same unit.

Relative standard deviation
Generally, it is defined as RSD (%) defined by the following equation:

$$\mathrm{RSD}(\%) = \frac{100}{\overline{X}} \times \sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{X})^2}{n-1}}$$

x_i: Measured value,

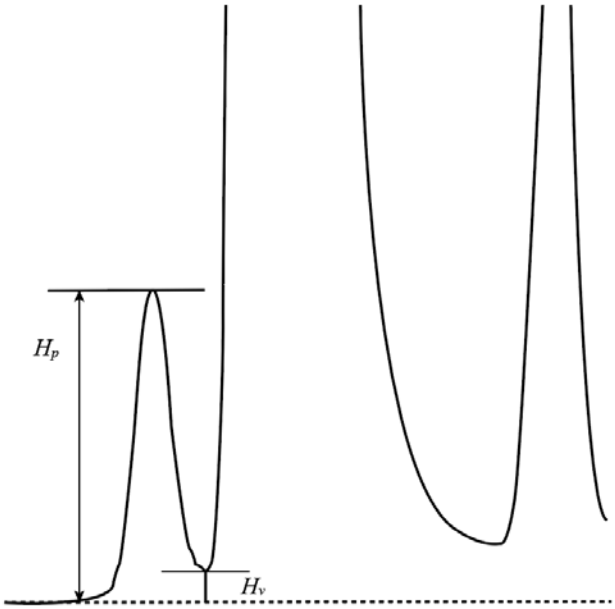
$\overline{X}$: Mean of measured values, and
n: Number of repeated measurements.

Complete separation of peak
Complete separation of the peak means that the resolution between two peaks is NLT 1.5.

Peak-valley ratio
It indicates the degree of separation between 2 peaks on a chromatogram when no baseline separation can be achieved. Peak-valley ratio is defined as *p*/*v* by the following formula:

$$p/v = \frac{H_p}{H_v}$$

H_p: Peak height from the baseline of the minor peak
H_v: Height from the baseline of the lowest point (peak valley) of the curve between major and minor peaks



Separation factor
The separation factor indicates the relation between the retention times of the peaks in the chromatogram and is defined as α in the following equation. The separation factor (α) is a characteristic indicator of thermodynamic difference in the distribution of two compounds. It is basically the ratio of not only their partition equilibrium coefficients or their mass distribution but also the retention times of the two compounds, and is determined from the chromatogram.

$$\alpha = \frac{(t_{R2} - t_0)}{(t_{R1} - t_0)}$$

t_{R1}, t_{R2}: Retention times of two compounds used for the resolution measurement where $t_{R1} < t_{R2}$
t_0: Time of passage of the mobile phase through the column (time measured from the time of injection of a compound with k=0 to the time of elution at the peak maximum).

Resolution
Resolution, R_S, indicates the relation between the retention time and the peak width of peaks in the chromatogram, and is

defined using the following equation:

$$R_S = 1.18 \times \frac{(t_{R2} - t_{R1})}{(W_{0.5h1} + W_{0.5h2})}$$

t_{R1}, t_{R2}: Retention times of two compounds used for measurement of the resolution where $t_{R1} < t_{R2}$

$W_{0.5h1}$, $W_{0.5h2}$: Peak widths at half peak height

where t_{R1}, t_{R2}, $W_{0.5h1}$ and $W_{0.5h2}$ have the same unit.

Number of theoretical plates

The number of theoretical plates is generally defined as N in the following equation to indicate the extent of band broadening of a compound in the column.

$$N = 5.54 \times \frac{{t_R}^2}{{W_{0.5h}}^2}$$

t_R: Retention time of compound

$W_{0.5h}$: Width of the peak at half peak height

where t_R and $W_{0.5h}$ have the same unit.

Note Standard analytes, internal standards, reagents or solvents should not contain any compounds that may interfere with the measurement.

Glass Containers for Injections
주사제용유리용기시험법

Glass containers for injections should not interact physically or chemically with the contained medicament to alter its property or quality, and should be able to protect the contained medicament from the invasion of microorganism through complete flame-sealing or other suitable methods. These shall conform to the following requirements. However, surface-treated containers for aqueous infusions are made from a material which meets the requirements for containers that are not flame-sealed as described in the Alkaline dissolution test in Method 1.

Containers for injections should be colorless or light brown and transparent and have no bubbles that interfere with the test described in Section L for Injections in the General Requirements for Pharmaceutical Preparations (II. 3. 3.1. L).

Containers intended for multiple doses are closed by rubber stoppers or other suitable closures. Stoppers should not physically or chemically interact with the contained medicament. When the needle is inserted, no fragments of the stopper should be mixed with the contents, and when the needle is pulled out, contamination from the outside should be prevented.

Containers intended for aqueous infusions are sealed by stoppers that meet the requirements for the test for rubber closure for aqueous infusions.

Alkaline dissolution test

This test is conducted through one of two methods according to the shape of the container and the intended use of the contained medicament.

Method 1 This method is applied to flame-sealed containers, or containers which are not flame-sealed, excluding those for aqueous infusions with a content of 100 mL or more.

Thoroughly rinse the inside and outside of the containers to be tested with water, dry, and crush if necessary. Place 30 – 40 g of the pieces in the steel mortar and crush it. Transfer the contents in the mortar to a No. 12 (1400 μm) sieve. Place the pieces retained on the sieve back into the steel mortar and repeat the operation until 2/3 of the sample passes through the No. 12 (1400 μm) sieve. Next, collect the portions that have passed through the No. 12 (1400 um) sieve, place them in No. 18 (850 μm) and No. 50 (300 μm) sieves, and shake them horizontally for 5 minutes with occasional light tapping. Take 7 g of the powder which passed through the No. 18 (850 um) sieve but not through the No. 50 (300 um) sieve. While placing the powder on the No. 50 (300 um) sieve, put it in a suitable container filled with water, rinse while gently shaking for 1 minute, rinse again with ethanol (95) for 1 minute, dry at 100 °C for 30 minutes, and cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder into a 20-mL hard Erlenmeyer flask, add 50 mL of water, and shake the flask gently to spread the powder evenly on the bottom of the flask. Cover the flask with a small hard beaker or a hard watch glass, heat it on a water bath for 2 hours, and cool to ordinary temperature. Transfer the solution in the flask into a 250-mL hard Erlenmeyer flask, thoroughly rinse the residue 3 times with 20 mL of water, and combine the washed solution in the 250-mL hard Erlenmeyer flask. Add 5 drops of bromocresol green-methyl red TS into the flask and titrate with 0.01 mol/L sulfuric acid until the color of the solution changes from green to slightly grayish blue and finally to pale grayish red purple. Perform a blank test through the same process and make any necessary correction.

The consumption of 0.01 mol/L sulfuric acid should not exceed the following volumes for the two container types.

Flame-sealed containers	0.30 mL
Not flame-sealed containers (including syringes used as containers)	2.00 mL

Method 2 This method is applied to containers that were not flame-sealed for aqueous infusions with a capacity of 100 mL or more.

Rinse the inside and outside of the container to be tested thoroughly with water, and dry it. Add water equivalent to 90% of the actual capacity of container, cover it with a small hard beaker or close it tightly with a suitable stopper, heat it in an autoclave at 121 °C for 1 hour, and leave it until it has cooled to ordinary temperature. Take exactly 100 mL of this solution, transfer it into a 250-mL hard Erlenmeyer flask, add 5 drops of bromocresol green-methyl red TS in the flask, and titrate with 0.01 mol/L sulfuric acid until the color of the solution changes from green to slightly grayish blue and finally to pale grayish red-purple. Separately, take exactly 100 mL of water, transfer it into a 250-mL hard Erlenmeyer flask, perform a blank test in the same manner, and make any necessary correction. At this time, the consumption of 0.01 mol/L sulfuric acid should not exceed 0.10 mL

Iron dissolution test for colored containers

Take at least 5 colored containers to be tested, rinse them thoroughly with water, dry them at 105 °C for 30 minutes, and add 0.01 mol/L hydrochloric acid corresponding to the indicated volume of each container. Seal the flame-sealed containers, and cover the not flame-sealed containers with small hard beakers or hard watch glasses. Then, heat them at 105 °C for 1 hour, and then cool down. Take exactly 40.0 mL of the solution to prepare the test solution according to Method 1 of the Iron limit test, and proceed with the test according to Method B. Prepare the control solution with 2.0 mL of iron standard solution.

Light-resistance test for colored containers

Take 5 colored containers to be tested and cut them into slices, with the surface of each piece as flat as possible. After cleaning the surface of the piece, fix it to the cell holder of a spectrophotometer so that light can pass perpendicularly through the center of the test piece, and measure the transmittance at wavelengths between 290 nm to 450 nm and 590 nm to 610 nm at intervals of 20 nm, using air as a reference. At this time, the percent transmission obtained between 290 nm and 450 nm should not be more than 50% and that obtained between 590 nm and 610 nm should not be less than 60%. However, for containers that were not flame-sealed with a wall thickness of 1.0 mm or more, the percent transmission at wavelengths between 590 nm and 610 nm should be more than 45%.

Arsenic

For a container that is not flame-sealed, rinse it with water at least twice, fill it with water, and allow to stand. Empty the container just before use and rinse it once with water and freshly boiled and cooled water within 20 – 25 minutes. Leave a flame-sealed container on a water bath or oven at about 50 ℃ for 2 minutes before opening it. Do not wash it after opening.

Add water equivalent to 90% of the actual capacity of the container and cover it with a small hard beaker or foil, which has been rinsed with freshly boiled and cooled water. Heat the container in an autoclave at 121 ℃ for 1 hour and cool it to ordinary temperature within 30 minutes. Take exactly 10.0 mL of this solution (if necessary, collect the solutions from multiple containers), mix it with 10.0 mL of hydrochloric acid and 5 mL of potassium iodide solution (1 in 5), heat the resulting solution on a water bath at 80 ° C for 20 minutes, and cool it. Add water to the cooled solution to make it up to exactly 100 mL and use it as the test solution. Take 0.1 mL, 0.5 mL, 1 mL, 1.5 mL, and 2 mL of the arsenic standard solution, and mix each of them with 10.0 mL of hydrochloric acid and 5 mL of potassium iodide solution (1 in 5) to prepare the standard solutions using the same process as the test solution preparation above. The content of arsenic in the test solution should be NMT 0.1 ppm, when tested according to the calibration curve method of Atomic Absorption Spectroscopy under the following conditions. At this time, a hydride generator is used for the test.

Gas: Acetylene - Air

Lamp: Hollow cathode lamp

Wavelength: 193.7 nm

Oxidizing agent for hydride generation

Hydrochloric acid

Reducing agent for hydride generation

Dissolve 5.0 g of sodium hydroxide and 2.5 g of sodium boron hydroxide in water to make 500 mL.

Arsenic standard stock solution

Weigh exactly 0.330 g of arsenic trioxide, dissolve it in 5 mL of sodium hydroxide solution (8.5 in 100), and add water to make it up to exactly 250.0 mL. Add water to make a 100-fold dilution. Prepare this solution immediately before use (10 ppm).

Arsenic standard solution

Add water to the arsenic standard stock solution to make a 10-fold dilution. Prepare this solution immediately before use (1 ppm).

Heavy Metals
중금속시험법

The Heavy Metals is a limit test of the quantity of heavy metal impurities contained in drugs. Heavy metal is a metallic mixture that is darkened by sodium sulfide TS under acidic conditions, and its amount is indicated in terms of the amount of lead (Pb).

In the monograph, the limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

Preparation of the test solutions and standard solutions

Unless otherwise specified, prepare test solutions and standard solutions using the following methods.

Method 1 Place the amount of sample specified in the monograph into a Nessler tube, and dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL and use it as the test preparation.

For the standard preparation, put the volume of the lead standard solution specified in the monograph into a Nessler tube and add 2 mL of dilute acetic acid and water to make a 50-mL solution.

Method 2 Weigh the amount of sample specified in the monograph, place it in a quartz or porcelain crucible, cover it loosely with lid, and carbonize it by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat the mixture carefully until the white fumes are no longer evolved, and then incinerate by ignition between 500 – 600 ℃. After cooling, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm the mixture for 2 minutes. Then, add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution turns a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings into a Nessler tube, and add water to make it up to 50 mL. Use the resulting solution as the test preparation.

For the standard preparation, evaporate 2 mL of nitric acid, 5 drops of sulfuric acid, and 2 mL of hydrochloric acid on a water bath and evaporate to dryness in a sand bath again. Moisten the residue with 3 drops of hydrochloric acid, then proceed in the same method as was used to prepare the test preparation. Then add the lead standard solution specified in the monograph and water to make it up to 50 mL.

Method 3 Weigh the amount of sample specified in the monograph, place it in a quartz or porcelain crucible, heat it gently and carefully, and then incinerate by ignition between 500 – 600 ℃. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then, add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution turns a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make it up to 50 mL. Use the resulting solution as the test preparation.

For the standard preparation, evaporate 1 mL of aqua regia to dryness on a water bath, and proceed in the same method as was used to prepare the test preparation. Then add the lead standard solution specified in the monograph and water to make it up to 50 mL.

Method 4 Weigh the amount of sample specified in the monograph, place it in a platinum or porcelain crucible, add and mix with 10 mL of magnesium nitrate in ethanol solution (1 in 10), ignite the ethanol to burn, and slowly heat to incinerate. After cooling, add 1 mL of sulfuric acid, heat it gently and carefully, and then incinerate by ignition between 500 – 600 ℃. If a carbonized substance remains at this time, moisten it with a small

amount of sulfuric acid and incinerate by ignition again. After cooling, dissolve the residue in 3 mL of hydrochloric acid, evaporate it to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warning. Add 1 drop of phenolphthalein TS, then add ammonia TS dropwise until the solution turns a pale red color. Add 2 mL of dilute acetic acid, and filter if necessary. Wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make it up to 50 mL. Use the resulting solution as the test preparation.

For the standard preparation, place 10 mL of magnesium nitrate in ethanol solution (1 in 10) and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, heat carefully, and then incinerate by ignition between 500 – 600 ℃. After cooling, add 3 mL of hydrochloric acid, and proceed in the same method as was used to prepare the test preparation. Then add the lead standard solution specified in the monograph and water to make it up to 50 mL.

Method 5 Unless otherwise specified in the monograph, weigh 0.3 g of extracts or 1.0 g of the fluid extracts, transfer it to a platinum or porcelain crucible, evaporate it to dryness on a water bath, and then incinerate by ignition between 500 – 600 ℃. After cooling, add 3 mL of dilute hydrochloric acid, warm, filter, and wash the residue twice with 5 mL of water each. Transfer the filtrate and washings to a Nessler tube, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the solution turns a pale red color. Add 2 mL of dilute acetic acid and water to make it up to 50 mL and use it as the test preparation.

For the standard preparation, take 3 mL of dilute hydrochloric acid and proceed in the same method as was used to prepare the test preparation. Add 3.0 mL of the lead standard solution and water to make it up to 50 mL.

Procedure

Add 1 drop each of sodium sulfide TS to both the test preparation and the standard solution, mix, and leave for 5 minutes. Compare the color of the two preparations by observing the top or the side of the Nessler tube on a white background.

The color of the test preparation must not be darker than that of the standard preparation.

Histamine
히스타민시험법

The Histamine is performed to detect histamine and histamine-like substances in a drug substance. Use the test conditions specified in the monograph. Unless otherwise specified, proceed as follows.

Standard solution of histamine

Accurately weigh an appropriate amount of histamine phosphate RS, dissolve it in water for injection or isotonic sodium chloride injection to make a solution which contains 1 μg of histamine ($C_5H_9N_3$) per mL, and use it as the histamine standard solution.

Test animals

Use healthy adult cats.

Sample and its amount

Sample is specified in the monograph. Unless otherwise specified, prepare the test solution by dissolving the sample in water for injection or isotonic sodium chloride injection. The test solution also can be prepared by suspending the sample in those solvents as long as it does not interfere with the test. If a solvent to use for the preparation of the test solution is specified in the monograph, a suspension is acceptable for the test provided that it does not interfere with the test. Unless otherwise specified, the amount of sample is 1.0 mL per kg of body weight of the test animal. The amount of sample specified in the monograph is the amount administered per kg of the test animal.

Procedure

Weigh the test animals, then inject phenobarbital, sodium hexobarbital or sodium pentobarbital intraperitoneally for general anesthesia. Expose the right carotid artery, completely dissect out all tissues around the vagus nerve using a scalpel and insert the cannula. Then, expose the femoral vein, operate a kymograph to record changes in blood pressure, and make sure that the blood pressure remains stable. Inject the histamine standard solution into the femoral vein as follows and determine the sensitivity of the test animals. Inject exactly 0.05 mL, 0.1 mL, and 0.15 mL of histamine standard solution per kg body weight at intervals of at least 5 minutes. Consider this injection as Series 1, and repeat the series of injections at an interval of at least 5 minutes. Discard the reading of the first Series 1. Stop the injection when the reduction of blood pressure caused by specific amounts of histamine injections is relatively constant, and use the reduction of blood pressure (2.67 kPa or more) caused by 0.1 μg/kg histamine as a standard for the test of the samples. Inject the amount of sample corresponding to the body weight of test animals into the femoral vein and observe the animals for 5 minutes. If a significant reduction in blood pressure occurs, retest with the histamine standard solution in the test animals, repeat the injections of the sample in the same manner described above, and confirm the results. If the test animal is sufficiently stable, it may be used for testing 2 or more samples.

Interpretation

The preparation complies with the test when the reduction of blood pressure caused by the test solution is less than that caused by 0.1 μg histamine per kg body weight.

Identification and Assay for Amino Acids
아미노산시험법

1. Identification Test

1) Thin Layer Chromatography

Method 1 Weigh 0.1 g of each amino acid reference standard, add water, and dissolve to make a solution of 100 mL. Use the resulting solution as the standard solution. Separately, dissolve a certain amount of the amino acid in water to make a concentration similar to that of the standard solution, and use the filtered solution as the test solution. Perform the test with these solutions following the thin-layer chromatography method. Apply 10 μL of the test solution and the standard solution onto a thin layer plate coated with silica gel for thin-layer chromatography (with a fluorescent indicator). Subsequently, develop the plate by moving it 10 cm using a developing solvent comprising a mixture of n-butanol, acetic acid (100), and water (2:1:1). Proceed to develop the plate for an additional 10 cm using a developing solvent consisting of a mixture of n-butanol and strong ammonia water (2:1). Afterward, heat the plate at 80 °C for 30 minutes, spray ninhydrin TS, and reheat the plate at 80 °C for 10 minutes. The test solution and the standard solution should exhibit spots with identical color and R_f value.

Method 2 Weigh 10.0 mg of each amino acid reference standard, add water, and dissolve to make a solution of 100 mL. Use the resulting solution as the standard solution. Separately, take a certain amount of the amino acid, dissolve it in water to make a concentration similar to that of the standard solution, filter it, and use the filtered solution as the test solution. Perform the test with these solutions using the thin-layer chromatography method. Apply 10 μL of the test solution and the standard solution onto a thin layer plate coated with cellulose for thin-layer chromatography. Subsequently, develop the plate by moving it 10 cm using a developing solvent comprising a mixture of n-butanol, acetone, water, and diethylamine (30:30:15:6). Proceed to develop the plate and additional 10 cm using a developing solvent consisting of either a mixture of isopropanol, water, and formic acid (80:20:4) or a mixture of butanol, water, dicyclohexyl amine (30:15:6). Next, heat the plate at 80 °C for 30 minutes, spray sulfuric acid TS or ninhydrin TS, and reheat the plate at 80 °C for 10 minutes. The test solution and the standard solution should exhibit spots with identical color and R_f value.

2) Liquid Chromatography

When tested according to the quantification method described below, each substance in the test solution should show a peak at the identical retention time as the corresponding substance in the standard solution.

3) Ultraviolet-visible Spectroscopy

When tested according to the quantification method described below, the test solution should show maximum absorbance at the same wavelength as the standard solution.

2. Quantification

1) Amino acids other than L-cysteine, N-acetyl-L-cysteine, N-acetyl-L-tyrosine, and L-tryptophan

a) Standard solution

Accurately weigh the reference standard for each amino acid and adjust the final concentration as specified. Use the resulting solution as the standard solution. However, if necessary, the concentration can be adjusted.

Substance Name	Concentration (μg/mL)	Substance Name	Concentration (μg/mL)
L-Aspartic acid	40	L-Threonine	35
L-Serine	32	L-Glutamic acid	44
L-Proline	70	L-Glycine (Aminoacetic acid)	26
L-Alanine	27	L-Cystine	10
L-Valine	35	L-Methionine	45
L-Isoleucine	40	L-Leucine	40
L-Tyrosine	55	L-Phenylalanine	50
L-Ornithine	40	L-Ornithine hydrochloride	50
L-Lysine	44	L-Lysine hydrochloride (L-Lysine acetate)	55
L-Histidine	47	L-Histidine hydrochloride	63
L-Arginine	52	L-Arginine hydrochloride	63
L-Asparagine	50	Aminoethyl sulfonic acid	30

b) Test solution

Accurately weigh a certain amount of the drug according to the labeled amount. Add 0.02 mol/L hydrochloric acid and shake to make a final concentration of each amino acid comparable to that of the standard solution. Subsequently, appropriately dilute or filter the solution and use it as the test solution.

c) Procedure

Measure the peak areas A_T and A_s by analyzing the test solution and the standard solution using liquid chromatography under the following conditions. However, note that the peak area of L-aspartic acid-L-ornithine is the combined sum of the peak areas of L-aspartic acid and L-ornithine in each solution.

$$\text{Amount of each amino acid (mg)} = \text{Final concentration of the standard solution of each amino acid (μg/mL)} \times \frac{A_T}{A_S} \times \frac{\text{Dilution factor}}{1000}$$

Operating conditions

Detector: Visible spectrophotometer (wavelength: 570 nm, except for proline and asparagine: 440 nm).

Column: A stainless steel column with an internal diameter of about 2.6 mm and a length of 15 cm is filled with ion-exchange resin for amino acid analysis.

Column temperature: Constant temperature around 53 ℃.

Reaction chamber temperature: Constant temperature around 98 ℃.

Prepare 6 types of buffer solutions (as shown in the table below, dissolve sodium citrate dihydrate in an appropriate amount of water, add sodium hydroxide sodium chloride, citric acid monohydrate, ethanol, benzyl alcohol, thiodiglycol, and BRIJ-35 solution. Shake the mixture, adjust the pH, and then add water to make a solution with a final volume of 1000 mL. Filter the resulting solution into a buffer storage bottle containing caprylic acid. Leave it overnight, and re-adjust the pH if necessary). Deliver the prepared buffer solutions in the order shown in the table below.

Note: Please refer to the table provided for specific details on the composition and order of the buffer solution.

	Buffer solution 1	Buffer solution 2	Buffer solution 3	Buffer solution 4	Buffer solution 5	Buffer solution 6
Usage	Mobile phase 1	Mobile phase 2	Mobile phase 3	Mobile phase 4	Sample dilution	Column regeneration
Sodium concentration (mol/L)	0.2	0.2	0.2	1.2	0.2	0.2
Water	700 mL	700 mL	700 mL	700 mL	700 mL	700 mL
Sodium citrate dihydrate	7.74 g	7.74 g	14.71 g	26.67 g	4.9 g	-
Sodium hydroxide	-	-	-	-	-	0.8 g
Sodium chloride	7.07 g	7.07 g	2.92 g	54.35 g	8.8 g	-
Citric acid monohydrate	20.00 g	20.00 g	10.50 g	6.10 g	35.0 g	-

Ethanol	130 mL	130 mL	-	-	-	-
Benzyl alcohol	-	-	-	5.0 mL	-	-
Thiodiglycol	5 mL	5 mL	5 mL	-	5 mL	-
BRIJ-35 solution	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL
pH	3.3	3.3	4.3	4.9	2.2	-
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL

Reaction reagent: Ninhydrin reaction TS

Flow rate of mobile phase: Adjust the flow rate to make the retention time of L-aspartic acid approximately 10 minutes (approx. 0.225 mL/min).

Selection of column: When performing a test with 50 μL of the standard solution under the aforementioned conditions, select the column that ensures the amino acids elute in the following order, with each peak being completely separated: aminoethylsulfonic acid, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, aminoacetic acid (L-glycine), L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine, L-histidine, and L-arginine.

2) L-cysteine (L-cysteine hydrochloride)

Accurately weigh an amount of sample equivalent to approximately 40 mg of L-cysteine (C3H7NO2S) or L-cysteine hydrochloride. Add 10 mL of 1 mol/L hydrochloric acid and heat the solution on a water bath for 15 minutes. Allow it to cool, then add water to make a solution of exactly 100 mL. Filter the solution, and take 10 mL of the filtrate. Add water to make it up to 100 mL, and use it as the test solution. Separately, accurately weigh 40 mg of L-cysteine or L-cysteine hydrochloride reference standard. Dissolve it in 50 mL of water and 10 mL of 1 mol/L hydrochloric acid. Add water to make it up to 100 mL. Take 10 mL of this solution, add water to make it up to 100 mL, and use it as the standard solution.

Take exactly 1 mL of both the test solution and the standard solution. Add 5 mL of Tris buffer (pH 8.0) and 1 mL of 5,5'-dithio-bis-(2-nitrobenzoic acid) TS, and then add water to make it up to 10 mL. Prepare a control solution by combining the test solution, standard solution, and 1 mL of water. Measure the absorbance A_T and A_S at a wavelength of 412 nm under the Ultraviolet-visible Spectroscopy.

Amount (mg) of L-cysteine ($C_3H_7NO_2S$)
= Amount (mg) of the reference standard of L-cysteine $\times \frac{A_T}{A_S}$

Amount (mg) of L-cysteine hydrochloride ($C_3H_7NO_2S.HCI.H_2O$)
= Amount (mg) of the reference standard of L-cysteine hydrochloride $\times \frac{A_T}{A_S}$

3) *N*-acetyl-L-cysteine

Accurately weigh a sample, equivalent to about 0.1 g of *N*-acetyl-L-cysteine ($C_5H_9NO_3S$), add 10 mL of 1 mol/L hydrochloric acid, and dissolve it in sodium bisulfite solution (1 in 2000) to make exactly 10 mL. Take 2 mL of this solution and combine it with 2 mL of the internal standard solution. Add sodium bisulfite solution (1 in 2000) to make exactly 50 mL, and use it as the test solution. Separately, accurately weigh about 0.1 g of *N*-acetyl-L-cysteine reference standard. Prepare it in the same manner as the test solution, and use it as the standard solution. For the test, take 5 μL of both the test solution and the standard solution, and conduct the Liquid chromatography under the following conditions. Calculate the ratio of the peak area of *N*-acetyl-L-cysteine to that of the internal standard in the test solution (Q_T) and the ratio of the peak area of *N*-acetyl-L-cysteine to that of the internal standard (Q_s) in the reference standard solution.

Amount (mg) of *N*-acetyl-L-cysteine ($C_5H_9NO_3S$)
= Amount (mg) of the reference standard of *N*-acetyl-L-cysteine $\times \frac{Q_T}{Q_S}$

Internal standard solution: Dissolve about 0.5 g of DL-phenylalanine in 100 mL of freshly prepared sodium bisulfite solution (1 in 2000).

Operating conditions

Detector: UV spectrophotometer (wavelength: 214 nm).

Column: A stainless steel column with an internal diameter of approximately 4 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with particle size ranging from 5 to 10 μm.

Mobile phase: Potassium dihydrogen phosphate solution (6.8 in 1000).

Column selection: The resolution I between *N*-acetyl-L-cysteine and the internal reference standard is NLT 6, and the relative standard deviation of the peak obtained from at least 5 injections of the standard solution is NMT 2.0%.

4) *N*-acetyl-L-tyrosine

Accurately weigh an amount of sample equivalent to approximately 25 mg of *N*-acetyl-L-tyrosine ($C_{11}H_{13}NO_4$) and add water to make a solution of exactly 100 mL. Take 5 mL of this solution, add water to make exactly 25 mL, and use it as the test solution. Separately, accurately weigh about 25 mg of *N*-acetyl-L-tyrosine reference standard and add water to make exactly 100 mL. Take 5 mL of this solution, add water to make it up to exactly 25 mL, and use it as the standard solution. Conduct the test using 20 μL of the test and standard solutions according to liquid chromatography under the following conditions. Measure the peak areas A_T and A_s in each solution.

Amount (mg) of *N*-acetyl-L-tyrosine ($C_{11}H_{13}NO_4$)
= Amount (mg) of the reference standard of *N*-acetyl-L-tyrosine $\times \frac{A_T}{A_S}$

Operating conditions

Detector: UV spectrophotometer (wavelength: 210 nm).

Column: A stainless steel column with an internal diameter of about 4 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with particle size ranging from 5 to 10 μm.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate solution and methanol (92: 8).

5) L-tryptophan

Accurately weigh an amount of sample equivalent to approximately 50 mg of test hermo-tryptophan ($C_{11}H_{12}N_2O_2$) and dissolve it in 70 mL of water. Add 2 mL of the internal standard solution and water to make it up to exactly 100 mL, and use it as the test solution.

Separately, accurately weigh about 50 mg of the L-tryptophan reference standard. Add 2 mL of the internal standard solution and water to make a solution of exactly 100 mL. Use this solution as the standard solution. Take 10 μL of both the test solution and the standard solution, and conduct the test using liquid chromatography under the following conditions. Calculate the peak area ratio Q_T and Q_S of L-tryptophan to the peak area of the internal standard in each solution.

$$\text{Amount (mg) of L-tryptophan } (C_{11}H_{12}N_2O_2) = \text{Amount (mg) of the reference standard of L-tryptophan} \times \frac{Q_T}{Q_S}$$

Internal standard solution—Dissolve about 100 mg of caffeine in 25 mL of water.

Operating conditions

Detector: UV spectrophotometer (wavelength: 210 nm).

Column: A stainless steel column with an internal diameter of about 4 mm and a length of 30 cm, filled with octadecylsilyl silica gel for liquid chromatography with particle size ranging from 5 to 10 μm.

Mobile phase: A mixture of methanol, water, and 0.005 mol/L sodium 1-heptanesulfonate solution (20: 80: 1).

Test solutions

1) Isatin-sulfuric acid TS: Weigh 11.4 g of isatin and dissolve in 100 mL of concentrated sulfuric acid.

2) BRIJ-35 solution: Weigh 25 g of BRIJ-35 (polyoxyethylene alcohol) and dissolve it in 100 mL of water by heating, if necessary.

3) Ninhydrin TS: Weigh 1 g of ninhydrin and dissolve it in 3% acetic acid/n-butanol solution to make 100 mL.

4) Ninhydrin reaction TS: Dissolve 82 g of anhydrous sodium acetate in 150 mL of water, add 25 mL of acetic acid (100), mix them, and add water to make 250 mL. Add 750 mL of methyl cellosolve to the resulting mixture and shake while passing nitrogen for about 20 minutes. Then, add 20 g of ninhydrin and perform the above procedure for about 15 minutes. Add 0.38 g of stannous chloride and continue the above procedure for about 10 minutes.

5) Tris buffer (pH 8.0): Add 1 mol/L hydrochloric acid to 100 mL of 0.2 mol/L tris (hydroxymethyl) aminomethane and adjust the pH to 8.0.

6) 5,5'-dithio-bis-(2-nitrobenzoic acid) TS: Dissolve 39.7 mg of 5,5'-dithio-bis-(2-nitrobenzoic acid) in 10 mL of ethanol. Then, add Tris buffer (pH 8.0) to 5 mL of this solution to make it up to 50 mL.

Inductively Coupled Plasma Spectrochemistry
유도결합 플라즈마 분석법

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) and Inductively Coupled Plasma-mass Spectrometry (ICP-MS) are elemental analysis methods in which inductively coupled plasma (ICP) is used as the excitation source (radio-frequency power) or the ionization source.

ICP is an excitation source composed of high-temperature argon plasma with intense thermal energy, which is formed by the high-frequency inductive coupling method. When a test solution is sprayed into the plasma, the atoms contained in the solution are excited. ICP-AES is the method used to measure the atomic emission spectrum of the light emitted from the plasma at that time, and to identify and quantitatively analyze the elements contained in the sample by determining the wavelength and intensity of its spectral lines.

Since ICP is also a good ionization source, the atoms in the test solution are ionized when the solution is sprayed into the plasma. ICP-MS is the method used to measure the mass spectrum of the element ions generated by ICP at that time by separating the ions by mass-to-charge (*m/z*) value and counting the intensities of ion peaks using a mass spectrometer as the detector.

When intense energy is added to an atom from the outside, the peripheral electrons of the atom would transit to an excited state by absorbing certain energy. The electron in the excited state would release the absorbed energy as light when it returns to its ground state. The light released at that time has a frequency v or a wavelength λ characteristic of each element, and the energy ΔE of the released light is expressed using the following equation.

$$\Delta E = hv = hc/\lambda$$

h: Planck's constant, *c:* Velocity of light

Since there are many excited states with various energy levels to which peripheral electrons might transit, many emission lines with various levels of energy can generate from one element, although some lines are strong and others are weak. However, there is only a limited number of emission lines observed in the ultraviolet/visible region and with enough detection sensitivity for the qualitative and quantitative analyses of each element. Since each element exhibits its own spectral line with a characteristic frequency (or wavelength) in the atomic emission spectrum, the elements contained in the test solution can be identified by determining the wavelengths of spectral lines in the spectrum detected via a spectrometer. Quantitative analyses of the elements in the test solution can also be performed by determining the intensity of the spectral line characteristic of each element. The elemental analysis method using this principle is ICP-AES.

ICP-MS is the elemental analysis method that is an alternative to optical analysis methods such as atomic absorption spectrometry and ICP-AES. In ICP-MS, the element ions generated by the ICP are separated by *m/z* value and the intensities of the separated ions are counted using a mass spectrometer. Compared to ICP-AES, ICP-MS is more sensitive, and with it an isotope analysis can be performed.

Both ICP-AES and ICP-MS can be used as excellent trace analysis methods specific to the inorganic impurities or coexisting elements in drug substances and drug products. Therefore, using these methods, qualitative and quantitative analyses can be performed not only for alkaline/alkaline-earth metals and heavy metals, but also for many elements for which adequate control is required to ensure the safety of pharmaceutical products. It would be useful for the quality assurance of drug substances to apply these methods to the profile analyses of inorganic elements contained in the substances, as these methods enable the simultaneous analysis of many elements.

1. Instruments

A. Instrument Configuration of ICP-AES

An ICP-AES is composed of an excitation source, a sample introduction port, a light emission unit, a spectroscope, a photometer and a data processor.

Excitation source The excitation source consists of a high-frequency power generator, a control circuit to supply and control the electric energy to the light emission unit, and a gas source. The sample introduction port, the main components of which are a nebulizer and a spray chamber, is used for introducing test solutions to the light emission unit after nebulizing the solutions.

Light emission unit The main components of the light emission unit, in which the elements contain in the test solution are atomized and excited to induce light emission, are a torch and a high frequency induction coil. The torch has a triple tube structure, and the test solution is introduced through the central tube. Argon gas is used to form the plasma and to transport the test solution. For the observation method of the light emitted from the light emission unit, there are two viewing modes: the lateral viewing mode in which the radial light of the plasma is observed, and the axial viewing mode in which the central light of the plasma is observed.

Spectroscope The spectroscope separates the light from the light emission unit to the spectral lines, and is composed of optical devices such as a light-converging system and a diffraction grating. There are two types of spectrometers: wavelength scanning spectrometers (monochromators) and simultaneously measuring spectrometers (polychromators) of the fixed-wavelength type. In addition, it is necessary to form a vacuum or to substitute the air in the chamber of the photometer with argon or nitrogen gas, when it is required to measure the spectral lines of the vacuum ultraviolet region (190 nm or shorter).

Photometer The photometer, which consists of a detector and a signal processing system, transduces the light energy of incident light to the electrical signal proportional to the intensity of the light. For the detector, a photomultiplier or a semiconductor detector is used.

Data processor The data processor is used to process the data obtained by the measurements, and it displays the calibration curves and measurement results.

B. Instrument Configuration of ICP-MS

An ICP-MS system is composed of an excitation source, a sample introduction port, an ionization port, an interface, an ion lens, a mass analyzer, an ion detector and a data processor. The excitation source, sample introduction port and ionization port have the same configuration as their counterparts in an ICP-AES system.

Interface The interface is the boundary component for introducing the ions generated by the plasma under atmospheric pressure into a high-vacuum mass analyzer, and is composed of the sampling cone and skimmer cone.

Ion lens The ion lens brings the ions introduced via the interface into focus and helps introduce the focused ions into the mass analyzer efficiently.

Mass analyzer For the mass analyzer, a common choice is a quadrupole mass analyzer. The interference caused by the polyatomic ions described later can be suppressed by placing a collision/reaction cell within the vacuum region before the mass analyzer, and introducing a gas such as hydrogen, helium, ammonia or methane into the cell.

Ion detector The ion detector transduces the energy of the ions that reached the detector to an electrical signal which is amplified by the multiplier. The data processor is used to process the data of the electric signal from the ion detector, and to display the calibration curves and measurement results, etc.

2. Pretreatment of Sample

When the samples to be analyzed are organic compounds such as pharmaceutical drug substances, they are usually digested and ashed using the dry ash method or the wet digestion method, and the test solutions for ICP-AES or ICPMS are prepared by dissolving the residues in small quantities of nitric acid or hydrochloric acid. When a sample is difficult to digest in the usual manner, the sample can be sealed in a closed, pressurized container and digested using microwave digestion equipment. Although liquid samples containing small amounts of organic solvents can be introduced directly into an ICP-AES or ICP-MS instrument without pretreatment, another alternative is introducing oxygen as the option gas to prevent any build-up of carbon generated from the solvent on the torch and the interface by contributing to the incineration of organic solvents.

3. Operation of ICP-AES

Set the argon gas flow at the specified rate and turn the high-frequency power source on to form the argon plasma. After confirming that the state of the plasma is stable, introduce a quantity of the test solution or the standard solution prepared using the method prescribed in the monograph into the instrument via the sample injection port, and measure the emission intensity of the analytical line specified for the element. When it is necessary to perform a Qualitative test to confirm or identify some elements, measure the emission spectrum in the wavelength range in which analytical lines are specified for the elements that appear.

A. Performance Evaluation of Spectrometers

Since each spectrometer requires its own calibration method based on its properties, a wavelength calibration must be performed according to the procedure indicated by the manufacturer.

For expressing the wavelength-resolving power of a spectrometer, the half height width of the analytical line in the emission spectrum of a specified element is usually defined in the form of “NMT xxx nm (a constant value).” The following emission lines, from the line with a low wavelength to that with a high wavelength, are usually selected for the above purpose: arsenic (As: 193.696 nm), manganese (Mn: 257.610 nm), copper (Cu: 324.754 nm) and barium (Ba: 455.403 nm).

B. Optimization of Operating Conditions

The operating conditions usually adopted are as follows. The operating conditions of the instrument should be optimized after stabilizing the state of the plasma by warming up the instrument for 15 ~ 30 min. The operating parameters should usually be set as follows: high frequency power, 0.8 ~ 1.4 kW; argon gas flow rate, 10 ~ 18 L/min for the coolant gas (plasma gas), 0 ~ 2 L/min for the auxiliary gas, and 0.5 ~ 2 L/min for the carrier gas. In lateral viewing mode, the point for measuring the light emitted from the plasma should be set within the range of 10 ~ 25 mm from the top edge of the induction coil, and the aspiration rate of the test solution should be set at 0.5 ~ 2 mL/min. In axial viewing mode, the optical axis should be adjusted so that the maximum value can be obtained for the intensity of the emission line measured. The integration time should be set within the range of one to several tens of seconds, taking the stability of the intensity of the emission line measured into account. When a test using an ICP-AES system is defined in a KP monograph, the operating conditions such as the analytical line (nm), high-frequency power (kW), and argon gas flow rate (L/min) should be prescribed in the monograph. However, it is necessary to optimize the operating conditions individually for each instrument and for each viewing mode used for the measurement.

C. Interference and Its Suppression or Correction

When discussing ICP-AES, the word “interference” is a

general term that indicates the influence of the coexisting components or matrix on the measurement results. Various interferences are roughly classified as either non-spectral interference (such as physical interference and ionization interference) or spectral interference. Their effects can be eliminated or reduced by applying the appropriate suppression or correction methods for the measurement.

Physical interference Physical interference means that the measurement results are influenced by the difference between the spray efficiencies of the test solution and the standard solution used for its calibration in the light emission unit, when the physical description (such as viscosity, density and surface tension) of the solutions differs. The effective methods for eliminating or reducing this type of physical influence are as follows. The test solution should be diluted to the level at which such interference will not occur; the properties between the test solution and the standard solution used for its calibration should be matched as much as possible (matrix matching method); and the internal standard method (intensity ratio method) or the standard addition method should be used.

Ionization interference Ionization interference is the influence due to the change in the ionization rate caused by the increase of electron density in the plasma, which is induced by a large number of electrons generated from the elements coexisting in the test solution at high concentrations. The suppression or correction method against ionization interference is essentially the same as the method used in the case of physical interference. The measurement conditions with low ionization interference can also be set by the selection and adjustment of the observation method of emitted light, the height for viewing, high-frequency power and carrier gas flow rate, and so on.

Spectral interference Spectral interference is the phenomenon which influences the analytical results of the sample by overlapping the various emission lines and/or the light with a continuous spectrum with the analytical line of the analyte element. To avoid this type of interference, it is necessary to select another analytical line which will not suffer from spectral interference. However, when no suitable analytical lines can be found, it is necessary to correct for spectral interference. In addition, when the pretreatment of the organic samples is not sufficient, the molecular band spectra (NO, OH, NH, CH, etc.) derived from nitrogen, oxygen, hydrogen and carbon remaining in the test solution might appear at the wavelength close to the analytical line of the analyte element, and could interfere with the analysis.

4. Operation of ICP-MS

After confirming that the state of the plasma is stable, optimize the instrument and confirm the system's suitability. Introduce a quantity of the test solution or the standard solution prepared using the method prescribed in the monograph, and determine the ion count numbers of the signal at the m/z value specified for the analyte element. When it is necessary to perform a test to confirm or identify some elements, measure the mass spectrum in the m/z value range specified for the analyte elements.

A. Performance Evaluation of Mass Spectrometer

The performance evaluation items for mass spectrometers are the mass accuracy and the mass resolving power. The mass accuracy should be adjusted by matching the *m/z* value of the mass axis of the mass analyzer to that of the standard element in the standard solution for the optimization specified in the operating conditions section of the monograph. With quadrupole mass spectrometers, it is preferable for the mass accuracy to be within ±0.2. For the mass resolving power, it is preferable that the peak width at 10% of the peak height in the observed ion peak be NMT 0.9.

B. Optimization of Operating Conditions

When a limit test or a quantitative test is performed, the sensitivity, background and generation ratio of oxide ions and doubly charged ions defined below should first be optimized to ensure that the performance of the instrument is suitable. For the optimization of operating conditions, the solutions of the elements which represent the low mass number elements, intermediate mass number elements and high mass number elements and are unlikely to be contaminated from the environment (e.g., ^{7}Li, ^{9}Be, ^{59}Co, ^{89}Y, ^{115}In, ^{140}Ce, ^{205}Tl and ^{209}Bi) are usually used as the standard solutions after adjusting to adequate concentrations.

Sensitivity The sensitivity is evaluated by the ion count numbers per second of integration time (cps). When a limit test or quantitative test is performed, it is preferable to have a sensitivity of several tens of thousands cps per 1 mg/L (ppb) for each element with a low mass number, intermediate mass number or high mass number.

Background For the background, it is preferable to be NMT 10 cps, when the measurement is performed at the *m/z* value at which no elements exist naturally (e.g., *m/z* value of 4, 8 or 220).

Generation ratio of oxide ions and doubly charged ions For the generation ratio of oxide ions and doubly charged ions, the numbers of oxide ions (e.g., ^{140}Ce: $^{140}Ce^{16}O^{+}$, *m/z* 156), doubly charged ions ($^{140}Ce^{2+}$, *m/z* 70) and monovalent ions ($^{140}Ce^{+}$, *m/z* 140) should be measured, and the generation ratios are calculated by dividing the ion count number of the oxide ions and doubly charged ions by that of the monovalent ions. It is preferable that the generation ratio of oxide ions (i.e., $^{140}Ce^{16}O^{+}/^{140}Ce^{+}$) be NMT 0.03 and that of doubly charged ions (i.e., $^{140}Ce^{2+}/^{140}Ce^{+}$) NMT 0.05.

C. Interference and their Suppression or Correction

In measurements using ICP-MS, attention must be paid to spectral interference and non-spectral interference.

Spectral interference Spectral interference includes isobaric interference and the interference caused by overlapping the mass spectrum of the analyte element with those of polyatomic ions or doubly charged ions. Isobaric interference is the interference by the isobaric element with the atomic mass adjacent to that of the analyte element– for example, the overlap of ^{40}Ar with ^{40}Ca and ^{204}Hg with ^{204}Pb. Since argon plasma is used as the ionization source, polyatomic ions such as $^{40}Ar^{16}O$, $^{40}Ar^{16}O^{1}H$, $^{40}Ar_2$ might be generated, and these would interfere with the measurements of ^{56}Fe, ^{57}Fe and ^{80}Se, respectively. When an instrument equipped with a collision/reaction cell is used, these polyatomic ions can be decreased in the cell. Doubly charged ions are the ions exhibiting their ion peaks at 1/2 the *m/z* value of the corresponding monovalent ions, and interference might occur when the element with an isotope with the mass number twice that of the analyte element might be present in the test solution.

Non-spectral interference Non-spectral interference includes not only the physical interference and the ionization interference, as in the case of ICP-AES, but also the matrix interference unique to ICP-MS. Matrix interference is the phenomenon in which the ion counts of every analyte element generally decrease when large amounts of other elements might coexist in the test solution. This tendency becomes more significant when the mass number of a co-existing element is larger and its concentration is higher, and when the mass number of the analyte element

is smaller. The extent of non-spectral interference can be estimated based on the recovery rate obtained by adding a known amount of the analyte element to the unknown sample. When it is found that the recovery rate is low and the reliability of the analysis is not assured, correction should be carried out using the internal standard method or the standard addition method. For ICP-MS in particular, the influence of non-spectral interference can be reduced by using the isotope dilution method.

5. System Suitability

When a limit test or quantitative test is performed using these methods, it is necessary to confirm that the performance of the instrument is suitable by carrying out a system suitability test in advance of the limit test or quantitative test, as defined below.

A. Evaluation for Required Detectability and Linearity

In an evaluation of an ICP-MS system for the required detectability and linearity, a solution is prepared in which the analyte element is not contained along with the standard solution with the concentration of the specification limit of the analyte element, and these solutions are used as the blank test solution and the solution for the system suitability test, respectively. The spectra obtained with these solutions are measured according to the test conditions optimized individually for each instrument, and it must be confirmed whether the emission line (or ion peak) of the analyte element is clearly observed at the specified wavelength (or m/z value) in the solution for the system suitability test when compared with the blank test solution. In this regard, the limit of the analyte element should be specified at the concentration of more than the quantitation limit (10σ). The test for required detectability is not required in the assay.

Linearity For the evaluation of linearity, it should be confirmed that the correlation coefficient of the calibration curve prepared by the procedure described in 6.A. Quantitative Analysis below is NLT 0.99. The confirmation of linearity is not required in quantitative analyses in section 6.A. or when isotope dilution as per section 6.A. is performed.

B. Evaluation for System Repeatability

Unless otherwise specified, when the test is repeated six times using the solution with the lowest concentration among those used for plotting the calibration curve according to the test conditions optimized individually for each instrument, it should be confirmed that the relative standard deviation of the observed values for the emission intensity (or ion count) of the analyte element is NMT the specified value (e.g., NMT 3% for an assay, and NMT 5% for a purity test).

6. Qualitative and Quantitative Analyses

A. Qualitative Analyses

In ICP-AES, the presence of the elements can be confirmed when the wavelengths and relative emission intensities of multiple emission lines from the test solution conform to those of the emission lines from the elements contained in the standard solution. In addition, the library of ICP-emission spectra attached to each instrument or the wavelength table of the spectra can also be used instead of the standard solution. Since the mass number region covering all the elements can be scanned in a short time in ICP-MS, the elements contained in the test solution can be analyzed qualitatively based on the m/z value of the ion peak in the mass spectrum obtained from the test solution. It would be feasible to list the metal catalysts and inorganic elements that might be contained in the sample as impurities, and for some elements (such as arsenic and lead) it might be necessary to monitor them in a routine manner from a safety perspective, and to carry out the profile of these inorganic impurities as a part of the manufacturing controls for a drug substance by referring to the representative emission lines of various elements shown in Table 1. In addition, the standard solution of each element should be prepared at an appropriate concentration considering the acceptance limit of each element to be specified separately.

Table 1. Representative emission lines of various elements (nm)

Al	396.153	In	230.606	Rb	780.023
As	193.696	Ir	224.268	Rh	233.477
B	249.773	Li	670.784	Ru	240.272
Ba	455.403	Mg	279.553	Sb	206.833
Be	313.042	Mn	257.610	Se	196.090
Cd	214.438	Mo	202.030	Sn	189.980
Co	228.616	Ni	221.647	Sr	407.771
Cr	205.552	Os	225.585	Tl	276.787
Cu	324.754	Pb	220.351	V	309.311
Fe	259.940	Pd	340.458	W	207.911
Hg	184.950	Pt	214.423	Zn	213.856

B. Quantitative Analyses

The quantitative analysis of an inorganic element in the test solution is usually performed using one of the following methods based on the emission intensity or ion count obtained by the integration of measurement data in a specified time.

1) Calibration curve method

Prepare standard solutions for plotting a calibration curve with different concentrations (four or more) of the analyte element. Using these standard solutions, the emission intensities at the analytical line specified for the analyte element by ICP-AES or the ion count numbers at the *m/z* value specified for the analysis of the element by ICP-MS are measured. The data obtained are then plotted against the concentrations, and this plot is used as the calibration curve. The concentration of the analyte element in the test solution is determined using this calibration curve.

2) Internal standard method

Prepare standard solutions for plotting a calibration curve with a fixed concentration of the internal standard element and different concentrations (four or more) of the analyte element. Using these standard solutions, the ratios of the emission intensities (or ion counts) of the analyte element to those of the internal standard element are determined. The data obtained are plotted against the concentrations, and this plot is used as the calibration curve. The internal standard element is also added to the test solution, so that the concentration of the internal standard element in the solution becomes the same as that in the standard solution. The concentration of the analyte element in the test solution is determined using the calibration curve plotted above. Before this method is applied, it is necessary to verify that the internal standard element to be added is not contained in the test solution. If the internal standard element to be added is present in the test solution, it is necessary to verify that the contaminated amount of standard element is negligible compared to the amount to be added. In addition, in ICP-AES, the following requirements must be met for the internal standard element: the changes in the emission intensity due to the measurement conditions and properties of the solution should be similar to those of the analyte element, and the emission line which does not cause spectral interference to the analytical line of the analyte element should be selected for the analysis. In contrast, in ICP-MS, it is preferable to select an internal standard element which does not cause spectral interference to the analyte element and has an ionization efficiency and

mass number equivalent to the analyte element.

3) Standard addition method

Take 4 portions or more of the test solution with the same volume, and prepare the following solutions: a solution in which the analyte element is not added; standard solutions for plotting the calibration curve (3 or more) in which the analyte element is added at different concentrations. Measure the emission intensities at the specified analytical line or the ion count at the specified *m/z* value for these solutions. Plot the obtained data against the concentrations calculated from the added amount of the analyte element. Calibrate the concentration of the analyte element in the test solution from the absolute value of the horizontal axis (concentration)-intercept of the regression line.

In ICP-AES, this method is useful for the correction of non-spectral interference caused by substances coexisting in the test solution, and it is applicable only to the cases in which spectral interference does not exist, or the background and the spectral interference are exactly corrected for and the relationship between the emission intensity and the concentration shows good linearity. In ICP-MS, this method is useful for the correction of non-spectral interference caused by coexisting substances in the test solution, and it is applicable only to the cases in which the spectral interference is exactly corrected for and the relationship between the ion count and the concentration shows good linearity down to the low concentration region.

4) Isotope dilution method:

The isotope dilution method is applicable only to ICP-MS. The concentration of the analyte element is determined from the change in the isotope composition ratio of the element by adding a substance containing a concentrated isotope with a known isotope composition that is different from the natural composition to the test solution. It is applicable only to the element which has two or more stable isotopes naturally and is able to perform the isotope analysis. A feature of this method is that the analytical precision is high and is not influenced by non-spectral interference, because the quantitation can be performed only by adding an adequate amount of a substance containing a concentrated isotope and measuring the isotope composition ratio of the test solution.

7. Note

Water and reagents and standard solutions used in this test are as follows.

1) Water water for an ICP analysis should be used. It should be verified prior to the test that the impurities contained in the water do not interfere with the analysis of the analyte element. Here, the water for an ICP analysis has an electrical conductivity of 1 $\mu S \cdot cm^{-1}$ or less (25 °C)

2) Reagents Reagents that are suitable for ICP analyses and are of high quality should be used.

3) Argon gas either liquefied argon or compressed argon gas with a purity of 99.99 vol% or higher should be used.

4) Standard solutions standard solutions should be prepared by diluting the Standard Solution (e.g., the Standard Solution defined in ISO guide 34, or a standard solution with a concentration certified by a public institution or scientific organization) to the specified concentration using the water for ICP analysis. However, in cases in which interference with the analysis might occur, it is preferable to match the properties of the standard solution to those of the test solution.

5) Preparation of a standard solution containing multiple elements a combination of the test solutions and elements should be selected so that precipitation and/or mutual interference do not occur.

Insoluble Particulate Matter in Injections
주사제의 불용성미립자시험법

The Insoluble Particulate Matter in Injections is a test method for insoluble particulate matter in injections and parenteral infusions consisting of mobile undissolved particles, other than gas bubbles, that are unintentionally present. The test is performed by using Method 1 (Light Obscuration Particle Count Test) or Method 2 (Microscopic Particle Count Test). Although Method 1 is preferably applied in most cases, it is necessary to test some preparations with Method 2 followed by Method 1. Not all injections can be tested by the two methods. In cases where Method 1 is not applicable, including emulsions, colloids, and liposomes with low transparency or high viscosity, the test is performed using Method 2. Similarly, for samples producing air or gas bubbles when drawn into the sensor, the use of the microscopic particle count test may be necessary. If the test cannot be performed properly due to the high viscosity of the injection, dilute with a suitable diluent to decrease the viscosity.

To ensure accurate estimation of particulate matter within the population, it is imperative to establish a statistically appropriate sampling plan for conducting this subset-based sampling test.

Method 1. Light obscuration particle count test

Apparatus Use a suitable apparatus based on the light obscuration principle which can automatically measure the particle diameter and the number of particles in each diameter. Verification of calibration, sample volume accuracy, sample flow rate, and counting accuracy should be performed at least once a year. The apparatus is calibrated using dispersions of spherical particles of known sizes between 10 μm and 25 μm with an appropriate certified reference material. These standard particles are dispersed in the water for the particulate matter test. Care must be taken to avoid aggregation of particles during dispersion.

Calibration Particles to be calibrated should be subject to particle size sensitivity measurement, using spherical polystyrene-based monodisperse particles (PSL particles) at least 5 μm, 10 μm, and 25 μm in diameter. Monodisperse particles have either domestic or international traceability in terms of length, and the relative standard deviation of the particle size is not greater than 3%. The particles for calibration are dispersed in water for the particulate matter test.

Manual method Particle size sensitivity is determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should be ± 20% of the measuring particle diameter. After measuring the sensitivity of the designated particle size, the particle diameter response curve is prepared using the method specified for the apparatus from the particle sensitivity measuring point. The threshold voltage of 5 μm, 10 μm, and 25 μm of the apparatus is obtained.

Electronic method In the use of a multichannel peak height analyzer, the particle size sensitivity is measured according to the half count method of the moving window system, the same as in the manual method, and the particle diameter response curve is prepared using the method specified for the apparatus. Then, the threshold voltage of 5 μm, 10 μm, and 25 μm of the apparatus is obtained. In this case, it should be verified that the same results as the manual method were obtained.

Automated method The particle diameter response curve of the apparatus can be obtained using the software in the apparatus. However, it should be verified that the same results as

the manual method are obtained.

Sample volume accuracy The accuracy of the sample volume is within NMT 5% of the measured volume when 10 mL of the test solution is measured, and the decrease of the test solution is measured using the mass method.

Sample flow rate The flow rate of the sample injected into the sensor is calculated from the measured volume and measurement time, and it should be confirmed that the calculated value is within the range specified for the apparatus.

Precision of sensor count rate The counting rate and particle diameter resolution of the particle-detecting sensor may vary depending on the parts precision and assembly precision of each sensor, even in the same type of sensor.

Accuracy of the sensor count rate It should also be validated by testing the particle diameter resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PS spheres with a mean diameter of approximately 1 μm, a concentration at 1000 particles/mL ± 10%, and relative standard deviation of NMT 5%). During measurement, stirring should be performed to ensure the uniformity of the sample.

Particle diameter resolution Measurement is performed using one of the following methods: a) Manual method to obtain the spread of histogram prepared from the counting values of the apparatus, b) Electronic method to obtain the spread of histogram by classifying the response signal of the apparatus using a multichannel peak height analyzer, or c) Automated method to obtain the spread of histogram of the response signal of the test particles using the software.

The difference between the threshold of particle size counting 16% and 84% of the total counts and the test particle size is NMT 10%. The electronic method and automated method are both validated as obtaining the same results as that from the manual method.

Count rate From counting particles of 5 μm or greater, it should be 763 – 1155 particles per 1 mL.

Accuracy of threshold set The threshold particle diameter counting 50% of the counting particles of 5 μm or greater should be within ± 5% of the average particle diameter of the test particles.

General precautions The test is performed in a clean cabinet, if possible, under conditions to limit particulate contamination.

Wash the filtration equipment and glassware, excluding the membrane filters, thoroughly with warm water and detergent, and then rinse with abundant amounts of water to ensure no detergent remains. Also, rinse the inside and outside of the apparatus with water for particulate matter test immediately before use. Take care not to introduce air bubbles when transferring some of the test injections to a container for measurement. Ensure that the environment is suitable for the test performing the following procedures with 5 mL of water for the particulate matter test, e.g., whether the glassware is properly cleaned and whether the water for the particulate matter test meets the specified regulations. For five measurements, the test environment is considered unsuitable if the number of particles 10 μm in size or larger exceeds 25 in 25 mL. The preparatory steps must be repeated until the environment, glassware, and water are suitable for the test.

Procedure Mix the contents of the sample by inverting the container slowly up and down 20 times successively. If there is sealing over the container opening, cautiously remove it. Remove air bubbles in the solution by allowing it to stand in the container for 2 minutes or by sonicating.

For large-volume injections (injections with a labeled amount of NLT 100 mL), perform the test for a single container. For small-volume injections of less than 25 mL, collect the contents of NLT 10 containers and put all of them in a clean container to make NLT 25 mL. If necessary, dilute with water for the particulate matter test to make 25 mL. If water for the particulate matter test is not suitable for the test, other suitable particle-free solvents can be used. For small-volume injections with a volume of NLT 25 mL (injections with a labeled amount less than 100 mL), perform the test for individual containers.

Powdered injections are dissolved in water for the particulate matter test. If water is not suitable for the particulate matter test, other suitable particle-free solvents can be used.

The number of samples must be adequate to enable proper statistical assessment. For large-volume or small-volume injections with a volume of at least 25 mL, perform the test for fewer than 10 containers according to an appropriate sampling plan.

With more than 25 mL of the test solution, test at least 4 portions with more than 5 mL of the test solution per portion, discard the first test result, and average the remaining test results to calculate the number of particles equal to or greater than 10 μm and 25 μm.

Evaluation For preparations supplied in containers with a labeled amount of NLT 100 mL, perform the evaluation according to the specification in Method 1 A). For preparations supplied in containers with a labeled amount of less than 100 mL, evaluate according to the specification in Method 1 B).

The preparation complies with the test when the average particle number is as specified below. If the result exceeds the limits, test the preparation according to Method 2, Microscopic Particle Count Test.

A) Large-volume injections (Injections with a labeled amount of NLT 100 mL): Not exceed 25 per mL equal to or greater than 10 μm, and not exceed 3 per mL equal to or greater than 25 μm

B) Small-volume injections (Injections with a labeled amount of less than 100 mL: Not exceed 6000 per container equal to or greater than 10 μm, and not exceed 600 per container equally to or greater than 25 μm

Method 2. Microscopic Particle Count Test

Apparatus Use a suitable binocular microscope, a filter assembly for retaining insoluble particulate matter, and a membrane filter. The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a movable stage capable of traversing the entire filtration area with a membrane filter, and an illuminator. It is adjusted to 100 ± 10 magnifications. The ocular micrometer is a lens with a circular diameter graticule (see Fig. 1) and consists of a large circle called the graticule field of view (GFOv), divided by crosshairs into quadrants, transparent and black reference circles 10 μm and 25 μm in diameters with 100 magnifications, and a linear scale graduated in 10 μm increments. When it is calibrated using a stage micrometer certified by a domestic or international standards agency, the relative error of the linear scale should be within ± 2%.

Illuminators Two types of illuminators are used. One is an episcopic brightfield illuminator in the microscope, and the other is an external, focusable auxiliary illuminator that provides reflected oblique illumination at an angle of 10° to 20°.

Filter assembly for retaining particulate matter The filter assembly consists of a filter holder made of glass or a material that does not interfere with testing, a membrane filter, and a suction device. The membrane filter is appropriately sized with or without a black or gray grid and has a pore diameter of 1.0 μm or less.

General precautions The test is performed in a clean

cabinet, if possible, under conditions to limit particulate contamination.

Wash the filtration equipment and glassware, except for the membrane filters, thoroughly with warm water and detergent, and then rinse with abundant amounts of water to ensure no detergent remains. Also, rinse the inside and outside of the apparatus with water for the particulate matter test immediately before use. Take care not to introduce air bubbles when transferring some of the test injections to a container for measurement. To evaluate the suitability of the test environment, such as whether the glassware is properly cleaned and whether the water for particulate matter test meets the specifications, perform the test as follows to check that the glass containers and membrane filters are properly cleaned, and the water for the particulate matter test is particle-free. Proceed as follows with 50 mL of the water for the particulate matter test to perform a test on the suitability of the test environment. If no less than 20 particles of no less than 10 μm in size or more than 5 particles of no less than 25 mm in size are present within the filtration area, it is evaluated that the test environment is not suitable. The preparatory steps must be repeated until the environment, glassware, and water are suitable for the test.

Procedure Mix the contents of the sample by inverting the container slowly up and down 20 times successively. If there is sealing over the container opening, cautiously remove it. Remove gas bubbles in the solution by sonicating.

For large-volume injections (injections with a labeled amount of no less than 100 mL), perform the test for a single container. For small-volume injections of less than 25 mL, collect the contents of no less than 10 containers in a clean container. If necessary, dilute with water for the particulate matter test to make 25 mL. If water is not suitable for the particulate matter test, other suitable particle-free solvents can be used. For small-volume injections with a volume of no less than 25 mL (injections with a labeled amount less than 100 mL), perform the test for individual containers.

Powdered injections are dissolved in water for the particulate matter test. If water is not suitable for the particulate matter test, other suitable particle-free solvents can be used.

The number of samples must be adequate to enable proper statistical assessment. For large-volume injections or small-volume injections with a volume of NLT 25 mL, perform the test for fewer than 10 containers according to an appropriate sampling plan.

Attach a membrane filter to the filter holder and wet the inside of the holder with a few mL of water for the particulate matter test. Slowly inject all the previously collected test solutions or all test solutions in a container using a funnel if necessary, and filter under reduced pressure. After the filtration, spray the water for the particulate matter test to wash the inner wall of the filter holder. Aspirate until the surface of the membrane filter is free of moisture. Transfer the filter to a Petri dish and allow it to air-dry with the lid slightly open. After air-drying, place the Petri dish on the stage of the microscope and count the number of particles equal to or greater than 10 μm in size and equal to or greater than 25 μm in size on all filters with reflected light. The number of particles in one field of view of the filter may be counted and the number of all particles on the filter can be calculated. Calculate the average number of particle count in the sample preparation. When particles are subdivided by size using a circular diameter graticule, the shape of each particle is considered a circle and is compared to the 10 μm and 25 μm reference circles. At this time, the particles should not be moved from their initial locations within the field of view scale, and not be superimposed on the reference circles. The size of white and transparent particles is measured by the internal diameter of the transparent circle, whereas the size of dark particles is measured by the outer diameter of the black circle.

In the microscopic particle count test, the size and the number are not measured for amorphous, semi-solid particles or morphologically indistinct particles that appear contaminated or discolored on the membrane filter. These materials have little or no surface irregularities and exhibit a gelatinous or film-like appearance. Method 1 (Light obscuration particle count test) may be suitable for measuring the number of particles of such materials.

Evaluation For preparations supplied in containers with a labeled amount of NLT 100 mL, evaluate according to the specification in Method 2 A).

For preparations supplied in containers with a labeled amount of less than 100 mL, evaluate according to the specification in Method 2 B).

The preparation complies with the test when the average particle number is as specified below.

A) Large-volume injections (Injections with a labeled amount of NLT 100 mL): Not exceed 12 per mL equal to or larger than 10 μm, and not exceed 2 per mL equal to or larger than 25 μm.

B) Small-volume injections (Injections with a labeled amount of less than 100 mL): Not exceed 3000 per container equal to or larger than 10 μm, and not exceed 300 per container equal to or larger than 25 μm.

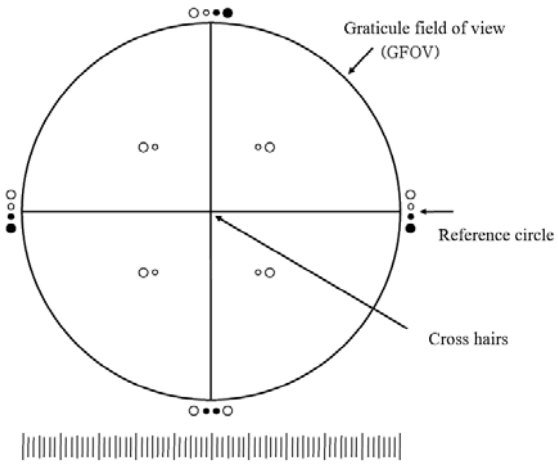


Figure 1. Circular diameter graticule

Insoluble Particulate Matter in Ophthalmic Solutions
점안제의 불용성미립자시험법

The Insoluble Particulate Matter in Ophthalmic Solutions is a limit test to quantify the size and the number of insoluble particulate matter in ophthalmic solutions.

Apparatus

A microscope, a filter assembly for collecting insoluble particulate matter, and a membrane filter for measurement.

1) Microscope

The microscope is equipped with an ocular photometer calibrated with an objective photometer, a movable stage, and an illuminator, and the magnification is adjusted to 100 times.

2) Filter assembly for collecting insoluble particulate matter

The filter assembly for collecting insoluble particulate matter consists of a filter holder made of glass or a material that does not interfere with the test, and a clip. The filter can be used under reduced pressure by attaching a membrane filter for measurement with a diameter of 24 mm or 13 mm can be used.

3) Membrane filter for measurement

The membrane filter for measurement is white in color, 25 mm or 13 mm in diameter, NMT 10 μm in nominal pore size, and a grid of about 3 mm on one side. When conducting a preparatory testing, use a filter that does not have particulates larger than 25 μm on it. If necessary, the filter is washed with water for particulate matter test.

Reagent

Water for particulate matter test: Water prepared before use by filtering through a membrane filter with a pore size of 0.45 μm or less. It contains NMT 10 insoluble particulates of 10 μm or greater per 100 mL.

Procedure

1) Aqueous ophthalmic solutions

All operations should be carried out carefully in clean facilities or equipment with little dust. Attach the measuring membrane filter to the filter holder, fix it with a clip, wash the inside of the filter holder with water for the particulate matter test, and then filter under reduced pressure with 200 mL of water for the particulate matter test at a rate of 20 to 30 mL per minute. Aspirate until the water on the membrane filter disappears, remove the membrane filter, place it in a flat-bottomed petri dish, tilt the lid, and dry the filter sufficiently at 50 ℃ or less. After drying, place the petri dish on the stage of the microscope and illuminate it with an illuminator to align the grid of the membrane filter with the coordinate axes of the movable stage, and adjust it so that insoluble particles are clearly visible. While moving the movable stage, count the number of particles of 150 μm or more on the effective filtration surface, and ascertain that the number is NMT 1. In this case, The size of the particulate shall be the longest diameter.. Next, attach another membrane filter for measurement to the filter holder, fix it with a clip, and wet the inside of the filter holder with several mL of water for the particulate matter test. For the sample, clean the outside of the container, gently invert it several times and shake to mix, carefully open it, clean the outside of the nozzle, and then pour it into a measuring cylinder that has been previously well rinsed with water for particulate matter test. Repeat this process to prepare 25 mL of test solution. Inject the sample slowly along the inner wall of the filter holder and aspirate slowly so that the sample always remains on the filter. As for the viscous sample, dilute it in advance with water for particulate matter test or a suitable diluent, and then filter as described above. When the sample amount on the membrane filter becomes small, wash the inner wall of the filter holder with 30 mL of water for the particulate matter test or suitable diluent. Repeat this process 3 times with 30 mL each. Then, gently aspirate until water on the membrane filter disappears, remove the membrane filter, place it in a petri dish, tilt the lid, and dry it at 50 ℃ or less. After drying, place the petri dish on the stage of the microscope and operate the microscope in the same procedure as described above to count the number of insoluble particulates of 300 μm or more on the effective filtration surface. The size of the insoluble particulates shall be the longest diameter.

2) Ophthalmic solutions dissolved before use

Follow the same procedures as for the aqueous ophthalmic solutions. However, after dissolving in the attached solvent, the test solution should be 25 mL.

3) Suspension-type ophthalmic solutions

Follow the same procedures as for the aqueous ophthalmic solutions. However, take 25 mL of the sample in a container that has been previously rinsed with water for the particulate matter test, add an appropriate volume of a suspension-solubilizing solution or adequate dissolution solvent, shake to dissolve the suspended particles, and use it as the test solution. A membrane filter which is not affected by the solvent should be used.

4) Disposable ophthalmic solutions

Follow the same procedures as for the aqueous ophthalmic solutions. However, use 10 samples for the test and use a 13-mm diameter membrane filter and 4-mm diameter filter holder for retaining particulate matter.

Interpretation

The limit is NMT 1 insoluble particulate matter of 300 μm or more in 1 mL of the solution.

Iron
철시험법

The Iron determines the presence of iron in drugs. The limit is defined by the amount of iron (Fe).

In the monograph, the limit of iron (expressed as Fe) is described in ppm and indicated within parentheses.

Preparation of test solution and control solution

Unless otherwise specified, prepare the test solution and the control solution as follows:

Method 1 Weigh the sample amount specified in the monograph. Add 30 mL of acetic acid-sodium acetate buffer solution for the Iron (pH 4.5). If necessary, dissolve by heating to obtain the test solution.

For the control solution, take the quantity of iron standard solution specified in the monograph. Add 30 mL of the acetic acid-sodium acetate buffer solution for the Iron (pH 4.5).

Method 2 Weigh the sample amount specified in the monograph. Add 10 mL of dilute hydrochloric acid and, if necessary, dissolve by heating. Incorporate 0.5 g of L-tartaric acid and one drop of phenolphthalein TS. After adding ammonia TS dropwise until the solution turns pale red, reintroduce 20 mL of acetic acid-sodium acetate buffer solution for the Iron (pH 4.5) to finalize the test solution.

For the control solution, utilize the iron standard solution quantity mentioned in the monograph. Add 10 mL of dilute hydrochloric acid and follow the procedure as was done for the test solution.

Method 3 Place the specified sample amount from the monograph into a crucible. Moisten with a modest quantity of sulfuric acid. Start with gentle heating, then incinerate by ignition. After cooling, incorporate 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3). Evaporate to dryness on a water bath. To the residue, add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. Heat to dissolve

and then introduce 30 mL of acetic acid-sodium acetate buffer solution for the Iron (pH 4.5).

For the control solution, add the iron standard solution amount specified in the monograph into a crucible. Add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3). Evaporate to dryness on a water bath and follow the procedure used for the test solution. Be sure to use a quartz or porcelain crucible that has been immersed in boiling dilute hydrochloric acid for 1 hour, rinsed thoroughly with water, and dried.

Method 4 Pour the sample amount specified in the monograph into a 50 mL Nessler tube. Add water to make it up to 45 mL and mix with 2 mL of hydrochloric acid for the test solution. For the control solution, pour 1 mL of iron standard solution into a 50 mL Nessler tube. Add water to make it up to 45 mL, and blend with 2 mL of hydrochloric acid.

Procedure

Unless otherwise specified, follow one of the procedures below. Please note that either Method A or Method C can be employed instead of Method B if it is demonstrated that this variation does not affect the test result.

Method A Transfer the sample and control solutions into Nessler tubes. Add 2 mL of L-ascorbic acid solution (1 in 100), mix thoroughly, and let stand for 30 minutes. Introduce 1 mL of α,α'-dipyridyl ethanol solution (1 in 200), add water to make it up to 50 mL, and wait another 30 minutes. Compare the colors of the solutions against a white backdrop. The color of the test solution must not be darker than the control solution.

Method B Dissolve 0.2 g of ascorbic acid in both the sample and control solutions and wait for 30 minutes. Add 1 mL of α,α'-dipyridyl ethanol solution (1 in 200) and allow another 30 minutes. Then, introduce 2 mL of picric acid solution (3 in 1000) and 20 mL of 1,2-dichloroethane. Shake vigorously, isolate the 1,2-dichloroethane layer, and if required, filter through a funnel loaded with 5 g of anhydrous sodium sulfate on a cotton ball. Compare the colors of the solutions against a white backdrop. The color of the test solution must not be darker than the control solution.

Method C Add 50 mg of ammonium persulfate and 3 mL of 30% ammonium thiocyanate solution to the sample and control solutions. Compare the colors of the solutions against a white backdrop. The color of the test solution must not be darker than the control solution.

(For this method, prepare the sample and control solutions according to Method 4.)

Laser Diffraction Measurement of Particle Size
레이저 회절에 의한 입자 크기 측정법

The method is based on the ISO standards 13320-1(1999) and 9276-1(1998).

The laser light diffraction technique used for measuring particle-size distribution analyzes the diffraction pattern generated when particles are exposed to a beam of monochromatic light. Historically, early laser diffraction instruments were limited to scattering at small angles. However, in recent times, this technique has been expanded to include laser light scattering over a wider range of angles and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by single particles and scattering by clusters of primary particles, such as agglomerates or aggregates. Since most particulate samples contain agglomerates or aggregates and, the typical focus is on the size distribution of primary particles, clusters are usually dispersed into primary particles before measurement.

In this measurement method, for non-spherical particles, the technique assumes spherical particles in its optical model to obtain an equivalent sphere-size distribution. As a result, the obtained particle-size distribution may differ from those obtained by methods based on other physical principles (e.g. sedimentation, sieving).

This chapter provides guidance on the measurement of particles size distributions various dispersed systems (e.g., powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids) through analysis of angle-dependent light-scattering patterns. Specific requirements for particle size measurement of particular products are not addressed in this chapter.

Principle

A representative sample, dispersed in a suitable liquid or gas at an appropriate concentration is passed through a monochromatic light bream, typically a laser. The light scattered at various angles by the particles is measured by a multi-element detector. Numerical values representing the scattering pattern are recorded for subsequent analysis. These numerical values are then transformed using an appropriate optical model and mathematical methods to determine the volumetric particle-size distribution by classifying the discrete particle sizes into groups.

Instrument

The instrument is installed in an environment free from electrical noise, mechanical vibrations, temperature fluctuations, humidity or direct bright light.

An example of a configuration of a laser light diffraction instrument is shown in *Figure 1*, although other equipment configurations may be used.

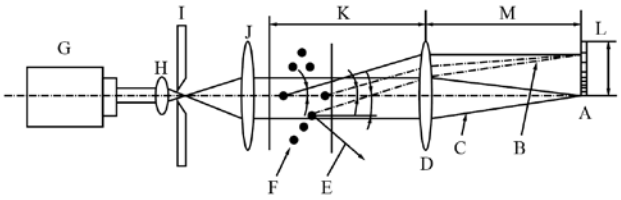


Figure 1. Example of a set-up of a laser light diffraction instrument

A. Obscuration detector
B. Scattered beam
C. Direct beam
D. Fourier lens
E. Scattered light not collected
F. Particle ensemble
G. Light source laser
H. Beam processing unit
I. Working distance of lens 4
J. Multi-element detector by lens 4
K. Focal distance of lens 4

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in two positions. In

the conventional case, the particles are detected within the working distance of the collecting lens as they enter the parallel beam. In the so-called reversed Fourier optics setup, particles enter behind the collecting lens, resulting in a converging beam. The advantage of the conventional setup is that it allows a reasonable path length for the sample within the working distance of the lens. The second setup allows only short path lengths but enables measurement of scattered light at larger angles, which is useful when dealing with submicron.

The interaction of the incident light beam and the ensemble of dispersed particles generates a scattering pattern with varying light intensities at different angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within certain limits, is not dependent on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is equivalent to the sum of the patterns from all individual single scattering particles positioned randomly. It's important to note that only a limited angular range of scattered light is collected by the lens(es) and, by the detector.

Development of the method

The measurement of particle size by laser diffraction can give reproducible data, even in the sub-micron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g. dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 µm to 3 mm. Because of recent advances in lens and equipment design, newer instruments are capable of exceeding this range routinely. With the validation report, the user demonstrates the applicability of the method for its intended use.

Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle-size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

Evaluation of the Dispersion Procedure

The sample to be analyzed is inspected, either visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted according to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as much as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method, it is highly advisable to check that comminution of the particles does not occur, and conversely, that particle or cluster dispersion is satisfactory. This can usually be achieved by changing the dispersing energy and monitoring the changes in the particle-size distribution. The measured size distribution should not change significantly when the sample is well dispersed, and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g. crystallization, milling) of the material has changed, the applicability of the method must be verified (e.g. by microscopic comparison).

Sprays, aerosols and gas bubbles in a liquid should be measured directly if their concentration is adequate, as sampling or dilution generally alters the particle-size distribution.

In cases of emulsions, pastes and powders from different dispersion systems, representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g. agitation, sonication) are often used for deagglomeration or deaggregate of clusters and stabilize dispersion. For these liquid dispersions, a recirculating system is most commonly used, consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing. Non-recirculating, stirred cells are useful when only small sample amounts are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols using suitable dry powder dispersers, which apply mechanical force for deagglomeration or deaggregation. Generally, these dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol, which is then blown through the measuring zone, typically into the inlet of a vacuum unit that collects the particles. However, for free flowing, coarser particles or granules, gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving, and the mass and percentage of the removed material should be reported. However, after pre-sieving, please note that the sample is no longer representative unless proven otherwise.

Optimization of the liquid dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must:

- be transparent at the laser wavelength and practically free from air bubbles or particles;
- have a refractive index that differs from that of the test material;
- be non-solvent of the test material (pure liquid or pre-filtered, saturated solution);
- not alter the size of the test materials (e.g. by solubility, solubility enhancement, or recrystallization effects);
- favor easy formation and stability of the dispersion;
- be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.);
- possess a suitable viscosity to facilitate recirculation, stirring and filtration.

Surfactants and/or dispersing agents are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH can assist in selecting a suitable dispersant.

A preliminary assessment of the dispersion quality can be performed through visual or microscopic inspection. Fractional samples can also be taken from out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore, a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

Optimization of the Gas Dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water and particles can be used. To remove contaminants from the compressed gas, a dryer with a filter may be used. Any vacuum unit should be located away from the measurement zone to prevent interference with the measurement.

Determination of the Concentration Range

In order to produce an acceptable signal-to-noise ratio in the detector, the particle concentration in the dispersion must exceed a minimum level, while staying below a maximum level to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. (Note: In different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g. obscuration, optical concentration, proportional number of total mass).

Determination of the Measuring Time

The time of measurement, detector reading time, and the acquisition frequency are determined based on the required precision. Typically, the measurement time allows for numerous detector scans or sweeps at short time intervals.

Selection of an Appropriate Optical Model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are occasionally applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the assumptions made about the test material (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.). If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly larger amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, since small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about 0.01-0.1 i) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g. shape, surface roughness and porosity), bear upon the final result.

Validation

Typically, the validity of a procedure may be assessed by the evaluating its specificity, linearity, range, accuracy, precision and robustness. In particle-size analysis using laser light diffraction ICH's definition of specificity is not applicable because it is not possible to differentiate between components in a sample, nor is it possible to distinguish agglomerates from dispersed particles unless complemented by microscopic techniques. The exploration of a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Instead of evaluating linearity, this method requires defining a concentration range within which measurement results remain consistent. Concentrations below that range result in errors due to a poor signal-to-noise ratio, while concentrations above that range produce errors due to multiple scattering. The range is mainly dependent on the instrument's hardware. Accuracy should be confirmed through appropriate instrument qualification and comparison with microscopy, while precision can be assessed through repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Specific limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to set acceptance criteria for repeatability such as% RSD $\leq$ 10% [n = 6] for any central value of the distribution (e.g. for x_{50}). Values at the sides of the distribution (e.g. x_{10} and x_{90}) should have less stringent acceptance criteria such as% RSD $\leq$ 15% [n = 6]. For particles below 10 µm, these values should be doubled. Robustness can be tested during the selection and optimization of the dispersion media and forces. Changes in the dispersing energy may be monitored by observing alterations in the particle-size distribution.

Measurement

Precautions

The instructions given in the instrument manual are followed:

- never look into the direct path of the laser beam or its reflections;
- earth all instrument components to prevent ignition of solvents or dust explosions;
- check the instrument set-up (e.g. warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight);
- in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent airflowin the case of dry dispersions; such effects can cause erroneous particle-size distributions.

Measurement of the Light Scattering of Dispersed Sample(s)

After proper aligning the optical part of the instrument, perform a blank measurement of the particle-free dispersion medium using the same method as that used for the measurement of the sample. Ensure that the background signal is below an appropriate threshold. Save detector data for later subtraction from sample data. Measure the sample dispersion according to the established method.

For each detector element, calculate an average signal, sometimes with its standard deviation. The magnitude of the signal from each detector element depends on the detection area, the light intensity and the quantum efficiency. The coordinates (size and position) of the detector elements, along with the focal distance of the lens determine, determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (blank measurement) indicates the proportion of scattered light and, therefore, the particle concentration.

Conversion of Scattering Pattern into Particle-Size Distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important, as most algorithms utilize mathematical solutions for scattering

from spherical particles. Furthermore, the measured data inevitably contain random and systematic errors that may affect the size distributions. Several mathematical procedures have been developed for use in available instruments. They include weighting deviations between measured and calculated scattering patterns (e.g. least squares), imposing constraints (e.g. non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each make and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

Replicates

The number of replicate measurements (with individual sample preparations) to be performed depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

Reporting of results

Typically, data on particle size distribution is recorded as cumulative undersize distribution and/or volume-based density distribution. The symbol x is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. $Q_3(x)$ denotes the volume fraction undersize at the particle size x. In a graphical representation, x is plotted on the abscissa and the dependent variable Q_3 on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as x_{10}, x_{50}, and x_{90} respectively) are frequently used. x_{50} is also known as the median particle size. It is recognized that the symbol d is also widely used to designate the particle size, thus the symbol x may be replaced by d.

Moreover, it is essential to include adequate information about the sample, sample preparation method, dispersion conditions, and cell type used. Since measurement results depend on the specific instrument, data analysis program, and optical model used, these details should also be documented.

CONTROL OF THE INSTRUMENT PERFORMANCE

Use the instrument according to the manufacturer's instructions and perform the necessary qualifications at an appropriate frequency, depending on the instrument and the substances being tested.

Calibration

Laser diffraction systems, while assuming idealized properties of the particles, are based on first principles of laser light scattering. Strict calibration is not essential, but it's crucial to ensure the proper functioning of the instrument. This can be achieved by using certified reference materials widely accepted in industrial practice. The entire measurement process, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It's vital to provide a comprehensive description of the entire operational procedure.

Preferred certified reference materials consist of spherical particles with a known mass-based size distribution, ideally certified by absolute methods. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

For a laser diffraction instrument to meet the requirements, the mean value of x50 from at least three independent measurements should not deviate by more than 3% from the certified range of values for the reference material. The mean values for x10 and x90 should not deviate by more than 5% from the certified range of values. Below 10 µm, these values should be doubled.

While using spherical particles for reference materials is preferred, non-spherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analyses performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same non-spherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials with stable particle-size distribution over time may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

Qualification of the System

In addition to the calibration, the performance of the instrument should be qualified at regular intervals or as often as needed, using appropriate reference materials as mentioned earlier.

The qualification of the system is based on the concept that the equipment, electronics, software and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in individual monographs, a laser diffraction instrument is considered to meet the requirements if the x_{50} value does not deviate by more than 10% from the range of values of the reference material. If values at the tails of the distribution are evaluated (e.g. x_{10} and x_{90}), these values should not deviate by more than 15% from the certified range of values. Below 10 µm, these values must be doubled.

NOTE: For calibration of the instrument, stricter requirements are laid down in the paragraph *Calibration*.

Liquid Chromatography
액체크로마토그래프법

The Liquid Chromatography is a method in which a sample mixture is injected into a column prepared with a suitable stationary phase, and liquid is used as the mobile phase to separate and analyze each component by using the difference in retention for the stationary phase. This method can be applied to liquid or soluble samples and is used for identification, purity, and quantification of a substance.

Each component of the mixture injected into the column is distributed in the mobile and stationary phases in a unique ratio, *k*.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

This ratio *k* is called the mass distribution ratio *k*' in liquid chromatography. Since there is the following relationship between this ratio *k*, hold-up time t_0 (time from the injection of a substance with $k = 0$ to the peak maximum of that substance) and the retention time t_R (time from the injection of the analyte to the peak maximum of that substance), the retention time becomes a unique value to the substance under the same conditions.

$$t_R = (1 + k)\ t_0$$

Apparatus

The apparatus usually consists of a pump system for the mobile phase, a sample injection port, a column, a detector, and a data collection device. Additional devices such as a mobile phase composition controller, a column thermostat, a pump for feeding reagents, a chemical reaction chamber can be used if necessary. Some pump systems can deliver the mobile phase and reaction reagents to a column and capillary tube at a constant flow rate. The sample injection port accurately introduces a certain amount of a sample into the instrument with good reproducibility. The column is made of inert metal or similar materials with a smooth inner surface, uniformly filled with packing materials for liquid chromatography of a certain size. Also, a column with a stationary phase chemically bound on the inner wall can be used instead of the packing materials. The detector is used to detect properties other than the mobile phase of the analyte, including ultraviolet absorption photometer, fluorescence photometer, differential refractometer, electrochemical detector, chemiluminescence detector, electrical conductivity detector, and mass spectrometer. It usually gives a signal proportional to the concentration of a sample of several μg or less. The recorder records the intensity of the signal caught by the detector. If necessary, a data processing device can be used to record or output chromatograms, retention times, and quantitative values of the analyte, etc. The mobile phase composition controller adopts stepwise control and concentration gradient control method and can control the composition of the mobile phase.

Procedure

After setting up the apparatus, use the detector, the column, and the mobile phase under the operating conditions specified in the monograph. Flow the mobile phase at the specified flow rate, and equilibrate the column to the specified temperature. Inject test solution or standard solution in the amount specified in the monograph through the sample injection port. Detect the separated analyte with a detector and obtain a chromatogram from a recording device. If the physical properties of the analyte, such as absorbance and fluorescence, are not suitable for detection by a detector, it is derivatized and detected accordingly. Derivatization is usually performed by pre-labeling or post-labeling methods.

Identification and purity test

In the Identification, check whether the retention times of the analyte in the test solution and the standard solution are the same and whether the peak shape of the analyte does not change even when the standard analyte is added to the test solution. In addition, when using a detector that can simultaneously detect the chemical structure of the analyte, the Identification can be performed with higher specificity by confirming that both the retention time and the information on the chemical structure match. A purity test is usually performed using a standard solution at a concentration equal to the impurity limit in the test solution or by the percentage peak area method. Unless otherwise specified, the isomer ratio of the test solution is determined by the percentage peak area method. In the percentage peak area method, the sum of the peak areas of each substance from the chromatogram is set to 100, and the composition ratio is determined from the peak area ratio of each substance. However, to obtain an accurate composition ratio, the peak area is corrected by the sensitivity coefficient for the active ingredient of the mixture.

Quantification

Internal standard method

In the internal standard method, choose a stable substance as an internal standard which has a retention time close to that of the analyte while ensuring complete separation from all peaks in the chromatogram. Prepare several standard solutions with stepwise concentrations by adding the reference standard of analyte to a certain amount of the internal standard specified in the monograph. From the chromatogram obtained by injecting a certain amount of these standard solutions, determine the ratio of the peak area or height of the reference standard of analyte to that of the internal standard. Create a standard curve by plotting this ratio as the vertical axis and the amount of reference standard of the analyte or the ratio of the amount of reference standard of the analyte to the amount of internal standard as the horizontal axis. This calibration curve is usually a straight line passing through the origin. Next, obtain a chromatogram under the same conditions as when creating a calibration curve by preparing a test solution with the same amount of the internal standard according to the method specified in the monograph. Determine the ratio of the peak area or height of the analyte to that of the internal standard and calculate the amount of the sample using the calibration curve. In the monograph, a standard solution that falls within the concentration range where the calibration curve is a straight line and a test solution with a concentration close to the standard solution are usually prepared. After injecting each amount specified in the monograph, perform a test using the liquid chromatography method to obtain the amount of the analyte under the same conditions.

Absolute calibration curve method

Prepare several standard solutions of the reference standard of analyte with stepwise concentrations and inject a certain amount of each standard solution accurately and reproducibly to obtain a chromatogram. Create a calibration curve from the obtained chromatogram by plotting the peak area or height of the analyte on the vertical axis and the amount of the reference standard of analyte on the horizontal axis. This calibration curve is usually a straight line passing through the origin. Next, prepare a test solution according to the method specified in the monograph, obtain a chromatogram under the same conditions as when preparing a calibration curve, measure the peak area or height of the analyte, and determine the amount of the sample from the calibration curve. In the monograph, a standard solution that falls within the concentration range where the calibration curve is a straight line and a test solution with a concentration close to the standard solution are usually prepared. After injecting each amount of those solutions specified in the monograph, perform a test using the liquid chromatography method to obtain the amount of the analyte under the same conditions. In this method,

all procedures, such as the injection procedure, should be performed under strictly fixed conditions.

Peak measurement method

The peak measurement method in Gas Chromatography is followed.

System suitability

The system suitability in Gas Chromatography is followed.

Precautions for changing the operating conditions

The following variables may be partially modified within the scope suitable for system suitability regulation: the internal diameter and length of the column, the particle size of packing materials, column temperature, the composition ratio of the mobile phase, the buffer composition of the mobile phase, pH of mobile phase, the concentration of ion-pairing reagent in the mobile phase, the salt concentration of the mobile phase, the number and time of mobile phase replacement, concentration gradient program and its flow rate, the composition and flow rate of derivatization reagent, the flow rate and reaction time of mobile phase, and the temperature of a chemical reactor.

Terminology

The definitions of terms in Gas Chromatography are followed.

Note

Standard analyte, internal standards, reagents, or test solutions should not contain substances that may interfere with the measurement.

Loss on Drying
건조감량시험법

The Loss on Drying measures the loss in mass of the sample, when dried under the conditions specified in the monograph. This method is applied to measure the amount of moisture, all or part of the water of crystallization, and volatile substances in the sample which are removed during the drying.

For example, a description of “NMT 1.0 percent (1 g, 105 °C, 4 hours) loss on drying” means that the loss in mass is NMT 10 mg per 1 g of this drug in the test in which about 1g of this drug is accurately weighed and dried at 105℃ for 4 hours, while specifying “NMT 0.5% (1 g, reduced pressure, phosphorus oxide, 4 hours) loss on drying” means that the loss in mass is NMT 5 mg per 1 g of this drug, in the test in which about 1 g of this drug is accurately weighed and dried under reduced pressure for 4 hours in a desiccator using phosphorus oxide as a desiccant.

Procedure

Accurately weigh a weighing bottle that has been dried for 30 minutes in advance using the method specified in the monograph. Take the sample within the range of ± 10% of the amount specified in the monograph and put it in a weighing bottle. Unless otherwise specified, spread it so that the height of the layer is less than 5 mm, and then weigh its mass accurately. Put this bottle in a drying chamber and dry it under the conditions specified in the monograph. If the sample is a large lump, crush it quickly to a size no larger than 2 mm in diameter, and use it for the test. After drying, take the weighing bottle out of the drying chamber and weigh its mass accurately. When the sample is dried by heating, heat and dry it at the temperature of ±2 ℃ as specified in the monograph, and allow to stand in the desiccator (silica gel) to cool down.

If the sample melts at a temperature lower than that specified in the monograph, expose the specimen for 1 to 2 hours to a temperature between 5 °C to 10 °C lower than the melting temperature, and then dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and replace it frequently.

Loss on Ignition
강열감량시험법

The Loss on Ignition is a method used to measure the loss in mass when the sample is ignited under the conditions specified in the individual monograph. This method is usually applied to inorganic drugs which lose a part of the components or impurities when ignited.

The description, for example, “40.0 - 52.0% (1 g, 450 - 550 °C, 3 hours)” indicates that the loss in mass is 400 mg to 520 mg per g of the sample in the test in which about 1 g of sample is weighed accurately and ignited at 450 °C - 550 °C for 3 hours.

Procedure

Ignite a crucible or a dish of platinum, quartz or porcelain at the temperature in advance as specified in the monograph so that the weight of the sample in it becomes constant. Then accurately weigh the sample after cooling.

Take the sample within the range of ± 10% of the amount specified in the monograph and transfer into the above container, and weigh it accurately. Ignite it under the conditions specified in the monograph, and leave in a desiccator (silica gel) to cool down, then weigh the mass accurately.

Melting Point
융점측정법

The melting point is typically defined as the temperature at which a crystalline substance melts upon heating, and the solid and liquid phases coexist in equilibrium. However, in practical terms, the melting point is defined as the temperature at the completion of melting, as observed by the state change during the heating process of the sample. The melting point serves as an indicator of the state, confirmation, and purity of a substance since it provides a unique value for each pure substance.

Melting points are measured using one of the following methods. Materials that are of relatively high purity and can be powdered are measured using Method 1, materials that are insoluble in water and challenging to powder are measured using Method 2, and petrolatum-based materials are measured using Method 3.

Unless otherwise specified, Method 1 is to be employed.

Method 1

Materials that are of relatively high purity and can be easily powdered typically use this method.

1) Apparatus

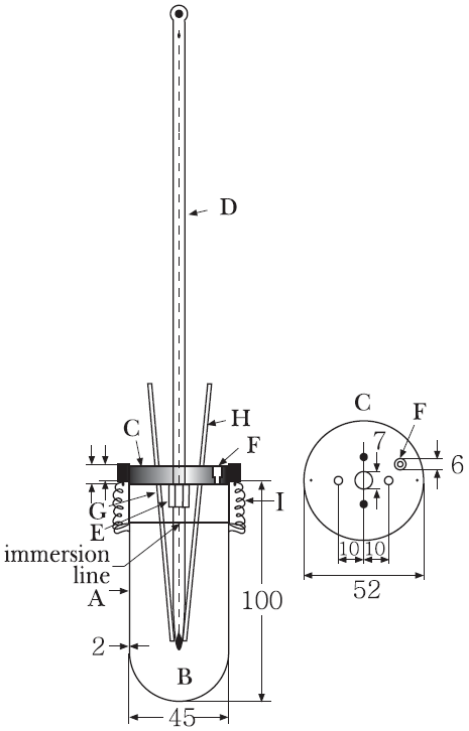
Use the apparatus shown in the figure.

However, apparatuses equipped with automated stirring, heating, and cooling functions may also be employed.

Bath fluid Use clear silicone oil with a kinematic viscosity ranging from 50 to 100 mm^2/s at ordinary temperature.

Acupuncture point thermometer There are No. 1–6 thermometers depending on the measurement temperature range. No. 1 is used for melting points less than 50 °C, No. 2 is for melting points between 40 °C and 100 °C, No. 3 is for melting points between 90 °C and 150 °C, No. 4 is for melting points between 140 °C and 200 °C, No. 5 is for melting points between 190 °C and 250 °C, and No. 6 is for melting points between 240 °C and 320 °C.

Capillary Use a one-end-closed capillary tube made of hard glass with an internal diameter of 0.8–1.2 mm, a length of 120 mm, and a wall thickness of 0.2–0.3 mm.



Figure

A: Heating vessel (made of hard glass)
B: Bath fluid
C: Lid (made of Teflon)
D: Acupuncture point thermometer
E: Thermometer fixture
F: Hole for adjusting the amount of bath fluid
G: Coil spring
H: Capillary
I: Lid holder

2) Procedure

Unless specified otherwise, grind the sample into a fine powder and then dry it in a desiccator containing silica gel for 24 hours. When referencing the post-drying stage, operate under conditions specified in the "loss on drying" section. Introduce the sample into a dry capillary tube H. Drop it into a glass column approximately 70 cm in length, placed on a glass or porcelain plate with the sealed end facing downward. Tap the tube to compact the sample, ensuring a layer thickness of about 3 mm. Heat the bath fluid B and gradually increase the temperature to about 10 °C below the anticipated melting point. Align the tip of the acupuncture point thermometer D with the meniscus of the bath fluid. Insert the capillary tube H containing the sample into the coil spring G, ensuring the sample-filled section is adjacent to the thermometer D mercury sphere's center. Increase the temperature at a rate of about 3 °C per minute. When the temperature is about 5 °C below the anticipated melting point, reduce the heating rate to 1 °C per minute. Once the sample in the capillary tube H has completely liquefied, record the reading of thermometer D as the melting point.

Apparatus suitability Regularly assess the apparatus's suitability using melting point reference standards. Melting point standards are prepared for apparatus suitability evaluation when using No. 2 to No. 5 thermometers: six types of high-purity substances (acetanilide, acetophenetidin, caffeine, sulfanilamide, sulfapyridine, and vanillin) with known melting points (MP_f, representing the temperature at the melting endpoint) can be chosen. If the melting point of the standard is gauged as per the procedure, choosing a suitable thermometer and standard based on the sample's expected melting point, the apparatus is deemed appropriate if the melting point for vanillin and acetanilide is within $MP_f \pm 0.5$ °C; for acetophenetidin and sulfanilamide, within $MP_f \pm 0.8$°C; for sulfapyridine and caffeine, within $MP_f \pm 1.0$°C. Perform this measurement thrice, with the average value serving as the melting point. If results seem unsuitable, revisit the procedure, ensuring proper sample filling, correct thermometer and capillary tube placement, appropriate bath fluid heating and stirring, and accurate temperature increase rate control. Should noncompliance persist despite correct conditions, recalibrate or replace the needlepoint thermometer.

Method 2

Apply to fat, fatty acid, paraffin, or lead.

1) Apparatus

Unlike in Method 1, use a beaker filled with water as both the bath fluid and heating container. Employ either an acupuncture point thermometer or a total immersion thermometer. Use the same capillary tube as in Method 1, but with both ends open.

2) Procedure

Carefully melt the sample at as low a temperature as possible. Draw the sample into a capillary tube to a height of about 10 mm, ensuring no bubbles enter. After securing the sample inside the capillary tube, allow to stand at NMT 10 °C for 24 hours or on ice for at least 1 hour. Attach the capillary tube to the thermometer using a rubber band, making sure the sample sits outside the center of the mercury sphere. Immerse the thermometer and attached capillary tube into a water-filled beaker, ensuring the sample's bottom end is 30 mm below the water's surface. Continuously stir while heating the water. When the temperature is 5 °C below the expected melting point, increase the rate to 1 °C per minute. Record the reading of the thermometer as the melting point when the sample begins to float in the capillary tube.

Method 3

Apply to petroleum-based materials.

1) Apparatus

Unlike in Method 1, use a beaker filled with water as both the bath fluid and heating container. Employ either an acupuncture point thermometer or a total immersion thermometer.

2) Procedure

Gently heat the sample to between 90–92 °C, stirring thoroughly until melted. Discontinue heating and let the sample stand at a temperature 8–10 °C above its melting point to cool. Cool

the thermometer (either type) to 5 °C, wipe and dry it, then immerse about half the mercury sphere into the sample. Immediately remove and position vertically to cool. Once the thermometer is marked or cloudy from the sample, immerse it in water below 16 °C for 5 minutes. Insert the thermometer into a test tube, using a cork stopper to maintain a 15 mm gap between the thermometer's bottom end and the test tube's base. Place this test tube in a beaker containing approximately 16 °C water. Heat the water, increasing the temperature by 2 °C per minute until the water bath reaches 30 °C, and then by 1 °C per minute thereafter. Record the temperature when the first droplet falls from the thermometer. Repeat this process three times. If the temperature differences across measurements are less than 1 °C, use the average as the melting point. If differences exceed 1 °C, repeat the procedure two more times and average all five readings to determine the melting point.

Microbial Assays for Antibiotics
항생물질의 미생물학적 역가시험법

The Microbial Assays for Antibiotics is a method used to determine the antimicrobial potency of antibiotics in drugs through microbiological methods. Unless otherwise specified, a test should be performed according to the following methods.

In assays specified in the monograph, the cylinder plate method is A), the standard curve method is B), and the Turbidimetric method is C). Follow the assay and the provisions stated in each monograph. Purified water, reagents, test solutions, gauges, and containers, etc. used in this test should be sterilized.

A) Cylinder plate method

(1) Cylinder

Use a stainless-steel cylinder with an outer diameter of 7.9 - 8.1 mm, an internal diameter of 5.9 - 6.1 mm, and a height of 9.9 - 10.1 mm that does not interfere with the test.

(2) Culture medium

Unless otherwise specified, use the medium with the following composition. However, if peptone is listed as a medium component, either meat peptone or Pancreatic Digest of Casein may be used. The pH of the medium should be adjusted as specified using 1 mol/L of sodium hydroxide TS or 1 mol/L of hydrochloric acid TS after sterilization. Medium is sterilized at 121 ℃ for 20 minutes using an autoclave. However, in the case of *Bacillus subtilis* ATCC 6633, the pH should be adjusted using ammonia TS, potassium hydroxide TS, or 1 mol/L of hydrochloric acid TS.

(A) Agar medium for seed and base layer

① *Bacillus subtilis* ATCC 6633

(A)

Peptone	5.0 g
Meat extract	3.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 7.8 - 8.0.

(B)

Peptone	5.0 g
Meat extract	3.0 g
Sodium citrate dihydrate	10.0 g
Agar 13.0 - 20.0 g	

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

② *Micrococcus luteus* ATCC 9341

(A)

Boiled meat peptone	6.0 g
Pancreatic Digest of Casein	4.0 g
Yeast extract	3.0 g
Meat extract	1.5 g
Glucose	1.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 7.8 - 8.0.

(B)

Peptone	6.0 g
Yeast extract	3.0 g
Meat extract	1.5 g
Glucose	1.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

③ *Staphylococcus aureus* ATCC 6538P

(A) Agar medium for strata

Peptone	6.0 g
Pancreatic Digest of Casein	4.0 g
Yeast extract	3.0 g
Meat extract	1.5 g
Glucose	1.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

(B) Agar media for base layer

Peptone	6.0 g
Yeast extract	3.0 g
Meat extract	1.5 g
Agar	13.0 - 20.0 g

Weigh the above materials, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

④ *Saccharomyces cerevisiae* ATCC 9763

(A)

Peptone	9.4 g
Yeast extract	4.7 g
Meat extract	2.4 g
Sodium chloride	10.0 g
Glucose	10.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.0 - 6.2.

⑤ *Escherichia coli* NIHJ and NCCP 14134

(A)

Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

⑥ Other organisms

(A)

Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

(B) Agar medium for transfer of test organisms

① *Saccharomyces cerevisiae* ATCC 9763

Peptone	5.0 g
Meat extract	2.0 g
Glucose	15.0 g
Sodium dihydrogen phosphate dihydrate	1.0 g
Magnesium sulfate	0.5 g
Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make

1000 mL, sterilize, and adjust the pH to 6.0 - 6.2.

② Other test organisms

(A)	Boiled meat peptone	6.0 g
	Pancreatic Digest of Casein	4.0 g
	Yeast extract	3.0 g
	Meat extract	1.5 g
	Glucose	1.0 g
	Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

(B)	Peptone	10.0 g
	Meat extract	5.0 g
	Sodium chloride	2.5 g
	Agar	13.0 - 20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

(C) Broth medium for suspension of test organisms

① *Saccharomyces cerevisiae* ATCC 9763

Peptone	10.0 g
Glucose	20.0 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 5.6 - 5.8.

② Other test organisms

Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g

Weigh the above ingredients, add purified water to make 1000 mL, sterilize, and adjust the pH to 7.0 - 7.1.

(3) Test organism

Use the test organism specified in the individual monograph.

(4) Preparation of test organism, test organism suspension or test spore solution

Prepare as specified in each monograph.. If *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 9637, *Escherichia coli* ATCC 10536, *Escherichia coli* NIHJ, *Escherichia coli* NCCP 14134, *Pseudomonas aeruginosa* NCTC 10490, *Saccharomyces cerevisiae* ATCC 9763, *Bacillus subtilis* ATCC 6633 and *Bacillus subtilis* ATCC 1768E are used as test organisms, prepare the test organism suspension or test spore solution by the following method, unless otherwise specified. Unless otherwise specified, thoroughly mix 0.5 - 2.0 mL of the test organism suspension prepared by the following (A), (B), (C) and (D) in advance and 100 mL of agar medium previously melted and cooled down to 48 °C to make the test organism suspension.

(A) Preparation of test organism suspension of Staphylococcus aureus ATCC 6538P, Staphylococcus epidermidis ATCC 12228, Micrococcus luteus ATCC 10240, Escherichia coli ATCC 9637, Escherichia coli ATCC 10536, Escherichia coli NIHJ, and Escherichia coli NCCP 14134

Inoculate the test organism in a slanted agar medium for transfer of test organism of A) (2) and (B) ② A with the test organism, culture it at 32 - 37 ℃ for 16 - 24 hours, and perform subculture at least 3 times. Inoculate this test organism in 9 mL of a slanted agar medium (internal diameter of test tube: 16 mm) for transfer of the test organism in A) (2) and (B) ② A with this test organism, and culture it at 32 - 37 ℃ for 16 - 24 hours. Add 10 mL of isotonic sodium chloride Injection to this slanted agar medium, collect the grown test organism, and suspend to prepare the test organism suspension. Store the suspension at a temperature not exceeding 5 ℃. Use the suspension of *Staphylococcus epidermidis* ATCC 12228 within 5 days, and use the suspension of the others within 7 days.

(B) Preparation of test organism suspension of *Pseudomonas aeruginosa* NCTC 10490 or *Micrococcus luteus* ATCC 9341

Inoculate the test organism in a slanted agar medium for transferring the test organism of A) (2) and (B) ② A, culture it as follows: at 25 - 26 ℃ for 40 - 48 hours for *Pseudomonas aeruginosa*; at 25 - 26 ℃ for 24 - 48 hours or at 32 - 37 ℃ for 16 - 24 hours for *Micrococcus luteus*. Perform subculture at least 3 times. Inoculate this test organism in about 9 mL of a slanted agar medium (internal diameter of test tube: 16 mm) for transferring the test organism of A) (2) and (B) ② A, culture it as follows: at 25 - 26 ℃ for 40 - 48 hours for *Pseudomonas aeruginosa*; at 25 - 26 ℃ for 24 – 48 hours or at 32 - 37 ℃ for 16 - 24 hours for *Micrococcus luteus*. Add 10 mL of isotonic sodium chloride injection to this slanted agar medium, collect the grown test organism, and suspend to prepare the test organism suspension. Store test organism suspension at a temperature not exceeding 5℃. Use the test organism suspension within 2 days after preparation for *Pseudomonas aeruginosa* and within 5 days, for *Micrococcus luteus*.

(C) Preparation of test organism suspension of *Saccharomyces cerevisiae* ATCC 9763

Inoculate the test organism in a slanted agar medium for transferring the test organism of A) (2) and (B) ①, culture it at 25 - 26 ℃ for 40 - 48 hours, and perform subculture at least 3 times. Inoculate this test organism in about 9 mL of a slanted agar medium (internal diameter of test tube: 16 mm) for transferring test organism in A) and (2) (B) ①, and culture at 25 - 26 ℃ for 40 - 48 hours. Add 10 mL of isotonic sodium chloride injection to this slanted agar medium, collect the grown test organism, and suspend to prepare the test microbial suspension. Store this test microbial suspension at a temperature not exceeding 5℃ and use within 30 days after preparation.

(D) Other Method

Culture the test organism of (A), (B) and (C) in the slanted agar medium for transferring test organism according to the above method, and suspend in about 3 mL of isotonic sodium chloride injection. Inoculate it onto the surface of 300 mL of the agar medium for transferring the test organism in a Roux culture bottle, spread evenly using a glass spreader, and culture at the specified temperature. Collect the grown test organism in an appropriate amount (usually about 50 mL) of isotonic sodium chloride injection to make a suspension. Add more isotonic sodium chloride injection to this suspension to make the test organism suspension. Unless otherwise specified, dilute this suspension with isotonic sodium chloride injection about tenfold to make the suspension so that the transmission ratio is 25% at 580 nm. In the case of *Pseudomonas aeruginosa* NCTC 10490, use the suspension without dilution.

(E) Preparation of test spore suspension of *Bacillus subtilis* ATCC 6633 and *Bacillus subtilis* ATCC 1768E

Suspend the test organism cultured in the agar medium for transferring the test organism in (A) (2) and (B) ② A in about 3 mL of isotonic sodium chloride injection solution, inoculate it onto 300 mL of a slanted agar medium for transferring test organism of (A) (2) and (B) ② B in a Roux culture bottle with the test organism, and spread it evenly using a glass spreader, and culture at 32 - 37 ℃ for more than 1 week to produce spores.

Suspend the spores in 100 mL of isotonic sodium chloride injection and heat at 65 °C for 30 minutes. Collect the spores by centrifugation, and again, wash them by centrifugation 3 times in about 50 mL of isotonic sodium chloride injection, suspend in sterile purified water or 100 mL of isotonic sodium chloride injection, heat again at 65 °C for 30 minutes, and store at a temperature not exceeding 5 °C. The spore suspension should be used within 6 months. Preliminarily determine the amount of the spore suspension added to 100 mL of the agar medium for seed layer by diluting it step by step, and choose a dilution to give a clear and definite zone of growth inhibition. in advance. Prepare agar media for the seed layer by mixing the decided amount of spore suspension and 100 mL of agar media previously melted and cooled down to 48 °C. Unless otherwise specified, usually add 0.1 ~ 1.0 mL of the spore suspension to 100 mL of agar media for seed layer.

(5) Preparation of cylindrical agar plate

Unless otherwise specified, dispense 20 mL of the melted agar medium for the base layer into each Petri dish with an internal diameter of 90 mm, and dispense 21 mL of the melted agar medium for the base layer into a Petri dish with an internal diameter of 100 mm. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden. Use the plates within the day of preparation. Dispense 4.0 mL of the seeded agar layer, which is specified in the individual monograph or (4) on an agar base layer plate in a Petri dish, spread evenly over the surface before hardening, and allow it to harden at ordinary temperature. Place 4 cylinders on an agar plate in a Petri dish so that the individual cylinders are equidistant from the center of the plate and equally spaced from one another (the cylinders are set on the circumference of a circle of 25 mm for a Petri dish with an internal diameter of 90 mm and 28 mm for a Petri dish with an internal diameter of 100 mm). In placing the cylinder, drop the cylinder vertically from a height of 10 ~ 13 mm.

(6) Standard solution

Use the solution of the standard product specified in the monograph. Hereinafter, the high-concentration standard solution is referred to as S_H, and the low-concentration standard solution is referred to as S_L.

(7) Test solution

It is specified in the monograph. Hereinafter, the high-concentration test solution is referred to as U_H, and low-concentration test solutions as U_L The test solution can be prepared within the range of ± 5% of the concentration specified in the monograph if it is clearly stated as such.

(8) Procedure

Unless otherwise specified, 5 cylindrical agar plates are grouped as one. Put S_H in the first cylinder and S_L in the second cylinder of each cylinder agar plate. Put U_H and U_L into the remaining two cylinders of each cylinder agar plate, respectively. Perform culture at 32 - 37 ℃ for 16 - 20 hours. After the culture, the diameter (mm) of each circular inhibition zone is measured within an accuracy of 0.5 mm.

(9) Calculation of potency

The following correlation is established between the potency (P) of the liquid in the cylinder and the diameter (d) of the inhibitory zone.

$$D = \alpha \times \log P + \beta \quad \text{(where, } \alpha \text{ and } \beta \text{ are constants)}$$

If necessary, using the above equation, the potency of the sample is calculated using the following formula:

Potency of the sample = A x Potency of S_H per mL x Dilution factor of U_H

$$\log A = \frac{I \times V}{W}$$

$$I = \log \frac{\text{potency of } S_H}{\text{potency of } S_L}$$

$$V = (\sum U_H + \sum U_L) - (\sum S_H + \sum S_L)$$

$$W = (\sum U_H + \sum S_H) - (\sum U_L + \sum S_L)$$

The sum of the inhibition zone diameters (mm) in each cylindrical plate of S_H, S_L, U_H and U_L is designated as $\sum S_H$, $\sum S_L$, $\sum U_H$ and $\sum U_L$, respectively.

B) Standard curve method

Unless otherwise specified, follow each of A) Cylinder Plate Method for the preparation of cylinder, culture medium, test solution, test organism suspension or test spore suspension. In addition, if the medium, etc. is specified in the monograph, comply with the provision in the individual monograph.

(1) Preparation of cylindrical agar plates

Follow the preparation of cylindrical agar plates as directed in A)(5). However, the difference is that a Petri dish with an internal diameter of about 100 mm is used, drop 6 cylinders are set on the circumference of a circle 28 mm, and the individual cylinders are spaced equally from one another with about a 60° interval.

(2) Standard solution

Use the standard solution specified in the individual monograph.

(3) Test solution

Use the test solution specified in the monograph. The concentration of the test solution specified in the individual monograph could be prepared within the concentration described ± 5%.

(4) Procedure

Use the concentrations of the standard solution and standard intermediate diluent specified in the individual monograph, and make a standard curve as follows. Use 3 cylinder-agar plates for each concentration of standard solution. Use 3 cylinder-agar plates as one assay set when Petri dishes are employed. Place 6 cylinders on each agar plate, apply a standard intermediate diluent to every other cylinder on each plate, and apply a concentration of standard solution to the remaining 3 cylinders. Perform this manipulation for each concentration of standard solution. Simultaneously, use 3 cylinder-agar plates for the test solution. Place 6 cylinders on each agar plate, apply a standard intermediate diluent to every other cylinder on each plate, and apply test solution to the remaining 3 cylinders. Incubate the plates at 32°C to 37 °C for 16 to 20 hours. After incubation, measure the diameters of the inhibition zone to an accuracy of 0.5 mm.

(5) Calculation of potency

Average the diameters (d) of the zone of inhibition from each concentration of the standard solution and standard intermediate diluent in 3 cylinder agar plates of every assay set, and average the diameters(d) of the zone of inhibition from standard intermediate diluent in all assay sets which is the average value for

correction. When the average values of standard intermediate diluent in each assay set are different from the average value for correction, make a correction by adding the difference to or subtracting the difference from the average value of each assay set. For example, if the average value of standard solution is 19.0 mm and the average value of standard intermediate diluent is 19.8 mm in an assay set, and the average value for correction is 20 mm, correct 19.0 mm to 19.2 mm (19.0 mm + (20.0 mm – 19.8 mm)). Generate the standard curve of log (potency) vs. diameter of zone of inhibition in semi-log paper, based on the corrected values. Then, average the diameters (d) of the zone of inhibition from the test solution and standard intermediate diluent in an assay set. If the average value of the test solution is larger than the average value of the standard intermediate diluent, make a correction by adding the difference value to the diameter of the zone of inhibition representing the center on the standard curve. If the average value of the test solution is smaller than the average value of the standard intermediate diluent, make a correction by subtracting the difference value from the diameter of the zone of inhibition representing the center on the standard curve. Estimate the potency of the test solution by extrapolating the diameter of zone of the test solution on the standard curve. Calculate the potency of the sample by multiplying the dilution factor. If the standard dilutions of five concentrations in geometric progression are used, calculate the L and H values from the following equations. Plot point L and point H on semi-log graph paper and construct a straight line for the standard curve.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

Where,

L: Calculated value of the diameter of the inhibition ring at the lowest concentration of the standard curve

H: Calculated value of the diameter of the inhibition ring at the highest concentration of the standard curve

c: Mean value for calibration

a: The corrected mean diameter of the inhibition ring at the lowest concentration standard solution,

b, d, e: The corrected mean diameter of each inhibition ring for each standard solution at high concentration in order.

Points L and H are marked on the semi-logarithmic sheet and are connected by a straight line.

C) Nephelometry

(1) Test organisms

Use *Klebsiella pneumoniae* ATCC 10031, *Staphylococcus aureus* ATCC 6538P, and *Escherichia coli* ATCC 10536 as test organisms, unless otherwise specified.

(2) Medium

Unless otherwise specified, use media with the following compositions. Use sodium hydroxide TS or 1 mol/L hydrochloric acid to adjust the pH of the medium to obtain the specified value after sterilization. Sterilize the media at 121 °C for 20 minutes in an autoclave.

(A) Agar medium for transferring test organisms

Peptone 6.0 g
Pancreatic Digest of Casein 4.0 g
Yeast extract 3.0 g
Meat extract 1.5 g
Glucose 1.0 g
Agar 13.0 - 20.0 g

Weigh the above materials, add purified water to make 1000 mL, sterilize, and adjust the pH to 6.5 - 6.6.

(B) Liquid medium for suspension of test organisms

Peptone 5.0 g
Yeast extract 1.5 g
Meat extract 1.5 g
Sodium chloride 3.5 g
Glucose 1.0 g
Dibasic potassium phosphate 3.68 g
Potassium dihydrogen phosphate 1.32 g

Weigh the above materials, add purified water to make 1000 mL, sterilize, and adjust the pH to 7.0 - 7.1. Instead of 3.68 g of dibasic potassium phosphate, 3.0 g of disodium hydrogen phosphate (anhydrous) can also be used.

(3) Preparation of test organism suspension

Inoculate the test organism onto the agar media for transferring test organisms, and incubate at 32 ~ 37 °C for 16 ~ 24 hours. The subculture should be performed at least three times. Inoculate the sub-cultured test organism onto slanted agar media for transferring the test organism, and incubate at 32 ~ 37 °C for 16 ~ 18 hours. Use the cultured organism to make a suspension of test organism as follows.

(A) Klebsiella pneumoniae ATCC 10031

Suspend the test organism in about 5 mL of sterile water, inoculate this suspension of the test organism onto the surface of 300 mL of the agar media for transferring test organisms in a Roux culture bottle, and spread evenly with glass spreader. Incubate at 32~37 °C for 16 ~ 24 hours. Scrape and suspend the resulting growth from the agar surface in an appropriate amount of sterile water to make the suspension of the test organism. Make the suspension so that the transmission ratio is 65% at 650 nm. Store the suspension at a temperature not exceeding 5 °C, and use within 14 days. Before use, add 6.0 mL of this suspension to 100 mL of the liquid media for suspending test organisms previously C, 2) (B) chilled to about 15 °C, and use the resulting suspension as the suspension of test organism.

(B) Staphylococcus aureus ATCC 6538P

Suspend the test organism in about 10 mL of the test organism suspension liquid medium, put in about 15 mL of liquid medium for suspension of test organisms, and use a spectrophotometer to ensure that a transmittance is about 85% at a wavelength of 650 nm. Put 4.0 mL of this solution into 100 mL of the medium of C) (2) (B) chilled to about 15℃ and use the resulting suspension as the test organism suspension.

(C) Escherichia coli ATCC 10536

Suspend the test organism in an appropriate amount of sterile purified water and ensure that the transmittance is 90% at a wavelength of 650 nm using a spectrophotometer. This liquid is stored below 5℃ and used within 14 days. Put 4.0 mL of this solution into 100 mL of the medium of C) (2) (B) chilled to about 15 °C and use the resulting suspension as the test organism suspension.

(4) Standard solution

Use the standard solution specified in the individual monograph.

(5) Test solution

Use the test solution specified in the individual monograph. The concentration of the test solution specified in the individual monograph could be prepared within the concentration described ± 5%.

(6) Procedure

Unless otherwise specified, proceed as follows: Distribute 1.0 mL of each concentration of the standard solution, the test solution, and water used as the blank, into each set composed of 3 test tubes (about 13.5 ~ 14.5 mm in internal diameter and about 130 mm in length). Add 9.0 mL of the suspension of the test organism to each tube, and then incubate on a water bath maintained at 35 ~ 37 °C for 3 ~ 4 hours. After incubation, add 0.5 mL of formaldehyde solution (1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

$$L = \frac{3a + 2b + c - e}{5}$$

(7) Calculation method

Average the transmittance or absorbance values of each concentration of the standard solution, the test solution and water used as the blank, respectively. Generate the standard curve based on the average values of transmittance or absorbance of each concentration of the standard solution, and estimate the potency of the test solution from its average value of transmittance or absorbance using the obtained standard curve. If the standard dilutions of five concentrations in geometric progression are used, calculate the L and H values from the following equation. Plot point L and point H on graph paper and construct a straight line for the standard curve.

$$H = \frac{3e + 2d + c - a}{5}$$

Where,

L: Calculated value of transmittance or absorbance for the lowest concentration of the standard curve

H: Calculated value of transmittance or absorbance for the highest concentration of the standard curve

a, b, c, d and e: Average transmittance or absorbance values for each standard dilution, where a is the value from the lowest concentration standard solution, b, c and d are values from each geometrically increased concentration standard solution, respectively, and e is the value from the highest concentration standard solution.

Microbiological Examination of Non-sterile Products
미생물한도시험법

The Microbiological Examination of Non-sterile Products includes the Total Microbial Count and the test for specified microorganisms. For the test, a mixture of several locations randomly selected on the product, or the formulation of a sufficient number of containers or parts is used as a sample. If samples are diluted with liquid medium, the test should be performed quickly. Also, precautions must be taken to prevent biohazards while performing the test.

I. Microbial Test of Non-Sterile Products: Viable Cell Count

1. Introduction

The purpose of this test is to quantitatively measure mesophilic bacteria and fungi which grow under aerobic conditions. The primary purpose of this test is to determine whether a substance or formulation complies with established microbial quality specifications. Perform the test as per the instructed method, including the number of samples to be taken, and interpret the results. This method is not applicable to products that contain viable microorganisms as active ingredients. If a procedure is equivalent to the tests specified in this chapter, alternative microbiological procedures including automated methods may be used.

2. General Procedures

Perform total viable aerobic count test under conditions designed to avoid extrinsic microbial contamination of the sample. The precautions taken to avoid contamination must not affect any of the microorganisms to be detected in the test. If the sample has antimicrobial activity, it should be removed or neutralized as much as possible. If inactivators are used for this purpose, make sure that their efficacy is confirmed, and that there is no toxicity for microorganisms. If surface-active substances are used for sample preparations, make sure that they have no toxicity for microorganisms and compatibility with inactivators.

3. Total Viable Count Methods

Usually, the membrane filtration method or plate-count methods are used. The most probable number (MPN) method is generally the least accurate method for microbial counts. However, it may be the optimal method for certain samples with a very low bioburden.

The appropriate method is selected based on factors such as the nature of the product and the required limit of microorganisms. The chosen method must allow testing of a sufficient sample size to judge compliance with the specifications and have the suitability confirmed.

4. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

4.1. General requirements

Confirm the ability of the test to detect microorganisms in the presence of samples. Then, confirm the suitability if there is a change in the test or the sample that may affect the result of the test.

4.2. Preparation of test strains

A standardized stable suspension or the method below is used to prepare a test strain. Manage microorganisms used for testing with a seed-lot system to ensure that they are NMT 5 passages removed from the original master seed-lot. Incubate each of the bacterial and fungal test strains separately as described in the conditions of Table I-1.

To prepare the suspension for test strains, use the buffered sodium chloride-peptone solution, pH 7.0 or phosphate buffer solution, pH 7.2. When suspending spores of *Aspergillus brasiliensis*, 0.05% of polysorbate 80 may be added to the buffer solution. Use the suspensions within 2 hours, or if stored at 2 - 8 °C, use within 24 hours of the preparation. Instead of preparing and diluting a fresh suspension of vegetative cells from *A. brasiliensis* or *Bacillus subtilis*, prepare a spore suspension for test inoculation. Each suspension may be stored at 2 - 8 °C for a validated period of time.

4.3. Negative control

To verify testing conditions, a negative control is performed with the diluent used in place of the test solution. There should be no growth of microorganisms. A negative control is

also performed when testing the sample as described in 5. A failed negative control should be investigated.

4.4. Growth promotion of the media

For ready-prepared media, perform the test for each batch. Test each batch of media prepared either from dehydrated and powdered medium or from the ingredients described. Inoculate a small number (NMT 100 CFU) of the microorganisms indicated in Table I-1 to soybean-casein digest broth and plates of Soybean-Casein Digest Agar medium and Sabouraud glucose agar medium. Separately incubate each strain under the conditions described in Table I-1 using broth or agar media. For agar media, the colony count of inoculums must be within 1/2 - 2 times the measured value of the standardized inoculum. For the test using freshly cultured microorganisms, make sure that the growth is equivalent to that previously obtained in the same type of medium where efficacy has been confirmed. For broth media, also make sure that the growth is equivalent to that previously obtained in the same type of medium where efficacy has been confirmed.

4.5. Suitability of the counting method in the presence of product

4.5.1. Preparation of the test solution

The method for the preparation of test solution depends on the physical characteristics of the sample to be tested. If none of the procedures described below can be performed, an alternative procedure must be established.

Table I-1. Preparation and use of test strains

Microorganism	Preparation of test strain	Growth promotion		Suitability of total viable count in the presence of products	
		Total aerobic microbial count	Total yeasts/mould count	Total aerobic microbial count	Total yeasts/mould count
Staphylococus aureus e.g., ATCC 6538, NCIMB 9518, CIP4.83, NBRC 13276 or KCTC 3881	Soybean casein digest agar medium or Soybean casein digest broth 30 - 35 ℃ 18 to 24 h	Soybean casein digest agar medium and Soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days		Soybean casein digest agar medium / MPN soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days	
Pseudomonas aeruginosa e.g., ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275 or KCTC 2513	Soybean casein digest agar medium or Soybean casein digest broth 30 - 35 ℃ 18 to 24 h	Soybean casein digest agar medium and Soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days		Soybean casein digest agar medium / MPN soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days	
Bacillus subsilis e.g., ATCC 6633, NCIMB 8054, CIP 52.62, NBRC 3134 or KCTC 1021	Soybean casein digest agar medium or Soybean casein digest broth 30 - 35 ℃ 18 to 24 h	Soybean casein digest agar medium or Soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days		Soybean casein digest agar medium / MPN soybean casein digest broth ≦ 100 CFU 30 - 35 ℃ ≦ 3 days	
Candida albicans e.g., ATCC 10231, NCPF 3179, IP 48.72, NBRC 1594 or KCTC 7965	Sabouraud glucose agar or Sabouraud glucose broth 20 - 25 ℃ 2 - 3 days	Soybean casein digest agar medium ≦ 100 CFU 30 - 35 ℃ ≦ 5 days	Sabouraud glucose agar ≦ 100 CFU 20 - 25 ℃ ≦ 5 days	Soybean casein digest agar ≦ 100 CFU 30 - 35 ℃ ≦ 5 days MPN: Not applicable	Sabouraud glucose agar ≦ 100 CFU 20 - 25 ℃ ≦ 5 days
Aspergillus brasiliensis e.g., ATCC 16404, IMI 149007, IP 1431.83, NBRC 9455, KCTC 6317 or KCTC 6196	Sabouraud glucose agar or Potato dextrose agar medium 20 - 25 ℃ 5 - 7 days, or until good sporulation is achieved	Soybean casein digest agar medium ≦ 100 CFU 30 - 35 ℃ ≦ 5 days	Sabouraud glucose agar ≦ 100 CFU 20 - 25 ℃ ≦ 5 days	Soybean casein digest agar ≦ 100 CFU 30 - 35 ℃ ≦ 5 days MPN: Not applicable	Sabouraud glucose agar ≦ 100 CFU 20 - 25 ℃ ≦ 5 days

4.5.1.1. Water-soluble products

Dissolve or dilute the product to be examined in buffered sodium chloride-peptone solution, pH 7.0, phosphate buffer solution, pH 7.2 or soybean casein digest broth (usually prepared by 10-fold dilution). If necessary, adjust to pH 6 - 8. Use the same diluents when further dilutions are necessary.

4.5.1.2. Nonfatty products insoluble in water

Suspend the product in buffered sodium chloride-peptone solution, pH 7.0, phosphate buffer solution, pH 7.2 or soybean-casein digest broth (usually prepared by 10-fold dilution). A surfactant such as 1 g/L of polysorbate 80 may be added to assist the suspension. If necessary, adjust to pH 6 - 8. Use the same diluents when further dilutions are necessary.

4.5.1.3. Fatty products

Dissolve the product in isopropyl myristate, sterilized by filtration, or if necessary, mix the product with the minimum amount of sterilized polysorbate 80 or another non-inhibitory surface-active substance, heated to below 40 ℃ (exceptionally below 45 ℃). If necessary, mix carefully while maintaining the temperature on a water bath. Add the selected pre-warmed diluent to make a 10-fold dilution of the sample. Mix carefully while maintaining the temperature for the shortest time necessary to

form an emulsion. With the diluent containing polysorbate 80 sterilized at an appropriate concentration or non-inhibitory surface-active substance, a 10-fold dilution series may be prepared.

4.5.1.4. Fluids or solids in aerosol form

Aseptically transfer the product into a membrane filter apparatus or into a sterile container for further sampling. Use either the total content or a certain amount sprayed a specified number of times in each sample container.

4.5.1.5. Transdermal patches

Remove the protective cover sheets (release liner) of the transdermal patches and place them, adhesive side upwards, on sterile glass or sterile plastic trays. Cover the adhesive surface with sterile porous material, for example, sterile gauze, to prevent the patches from sticking together. Select a diluent containing inactivators such as polysorbate 80 and/or lecithin, soak the patches into a suitable volume of the diluent, and shake vigorously for 30 minutes.

4.5.2. Inoculation and dilution

Add a sufficient amount of the test microbial suspension to obtain inoculum of NMT 100 CFU to a test and control solution (without sample) prepared in 4.5.1. The amount of the test strain suspension inoculated should not exceed 1% of the diluted amount.

4.5.3. Neutralization and removal of antimicrobial activity

As per the methods in 4.5.2. and 4.5.4., perform the test, and compare the number of microorganisms recovered from the test solution to that of the control solution. If the growth is inhibited (if the number of microorganisms recovered from the test solution is less than 1/2 of the control solution), modify the procedure for the Total Microbial Count to ensure the efficacy of results. Modification of the procedure may include the following methods: (1) an increase in the volume of the diluent or medium, (2) an incorporation of a specific or general neutralizing agents into the diluents, (3) membrane filtration or (4) a combination of the above measures.

Neutralizing agent

A neutralizing agent may be used to neutralize the activity of antimicrobial agents (Table I-2). The neutralizing agent can be added to the selected diluent or the medium. If possible, add it before sterilization. If a neutralizing agent is used, perform a blank test with only the neutralizing agent without the sample to ensure its efficacy and non-toxicity for microorganisms.

If a suitable neutralizing method cannot be established, assume that the failure to isolate the inoculum is attributed to antimicrobial activities of the sample. As such, it can be assumed that the possibility of the sample being contaminated by the same species of the inoculum or allied species is low. However, since there is a possibility that the sample inhibits only some of the microorganisms and not the strains other than the inoculum, perform tests with the lowest concentration suitable for microorganism growth and the accepted standards.

Table I-2. Potential neutralizing agents/method on interfering substances

Interfering substance	Neutralizing agents/ methods
Glutaraldehyde, mercurials	Sodium hydrogen sulfite (sodium bisulfate)
Phenolics, alcohol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary ammonium compounds (QACs), parabens, bis-biguanides	Lecithin
Quaternary ammonium compounds (QACs), parabens Iodine	Polysorbate
Mercurials	Thioglycollate
Mercurials, halogens, aldehydes	Thiosulfate
Ethylenediaminetetraacetic acid salt (EDTA)	Magnesium ion or calcium ion

4.5.4. Recovery of microorganism in the presence of product

Perform separate tests for each of the microorganisms listed in Table I-1. Count only the added microorganisms.

4.5.4.1. Membrane filtration method

Use membrane filters with a pore size of 0.45 μm or less. Select a type of filter material in which the bacteria-capture efficiency is not affected by components of the sample. Use 1 membrane filter for each of the microorganisms listed in Table I-1. Transfer a suitable amount of the sample prepared as described under 4.5.1. to 4.5.3. (preferably representing 1 g of the product, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent. For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of Soybean-Casein Digest Agar. For the determination of total combined yeasts/mould count (TYMC), transfer the membrane to the surface of the Sabouraud Dextrose Agar. Incubate the plates under the conditions specified in Table I-1, and measure the colony count.

4.5.4.2. Plate-count methods

Perform the test according to the plate-count methods at least in duplicate for each medium and use the mean count of the result.

4.5.4.2.1. Pour-plate method

When using a Petri dish 9 cm in diameter, add to 1 mL of the sample prepared as described in 4.5.1. to 4.5.3., Suitability of the Counting Method in the presence of product in 4.5., and 15–20 mL of Soybean-Casein Digest Agar or Sabouraud Dextrose Agar medium warmed at less than 45 °C. If larger Petri dishes are used, increase the amount of agar medium accordingly. For each of the microorganisms listed in Table I-1, use at least 2 Petri dishes. Incubate the plates as indicated in the conditions of Table I-1. Take the arithmetic mean of the microbial counts of each medium and calculate the number of colonies.

4.5.4.2.2. Spread plate method

When using a 9 cm diameter Petri dish, add 15 – 20 mL of Soybean-Casein Digest Agar or Sabouraud Dextrose Agar at about 45 °C to each Petri dish and allow to solidify. Dry the surface-spread media in a laminar flow cabinet or thermostat. If larger Petri dishes are used, increase the amount of agar medium accordingly. For each of the microorganisms listed in Table I-1 , use at least 2 Petri dishes. Take no less than 0.1 mL of the sample prepared as per 4.5.1. to 4.5.3., Suitability of the Counting Method in the presence of the products, and spread on the entire surface of medium. Incubate and count as prescribed under 4.5.4.2.1.

4.5.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the platecount method. In particular, when enumerating moulds, the reliability is low. For these reasons, the MPN method is used for TAMC counting when there is no other method available. When applying this method, proceed as follows. As described in 4.5.1. to 4.5.3., Suitability of the Counting Method in the presence of the products, prepare a series of at least 3 serial ten-fold dilutions of the product. Take 1 g or 1 mL at each level of dilution, and inoculate in 3 test tubes with 9 - 10 mL of soybean-casein digest broth. If necessary, a surfactant such as polysorbate 80, or an inactivator of anti-microbial agents may be added to the medium. Therefore, if 3-fold dilution series are prepared, it means that inoculation is performed in 9 test tubes. Incubate all test tubes at 30 – 35 °C for no longer than 3 days. If the reading of the result is difficult or uncertain due to the attributes of the sample, subculture in the same medium, or Soybean-Casein Digest Agar for 1 - 2 days at the same temperature and use these results. Calculate the most probable number of microorganisms per gram or milliliter of the sample from Table I-3.

Table I-3. Most probable number of microorganisms

Combinations of test tubes that show microorganism growth in each set			Most probable number of 1 g or 1 mL of the product	95% reliability limit
Number of g or mL per test tube				
0.1	0.01	0.001		
0	0	0	< 3	0 to 9.4
0	0	1	3	0.1 to 9.5
0	1	0	3	0.1 to 10
0	1	1	6.1	1.2 to 17
0	2	0	6.2	1.2 to 17
0	3	0	9.4	3.5 to 35
1	0	0	3.6	0.2 to 17
1	0	1	7.2	1.2 to 17
1	0	2	11	4 to 35
1	1	0	7.4	1.3 to 20
1	1	1	11	4 to 35
1	2	0	11	4 to 35
1	2	1	15	5 to 38
1	3	0	16	5 to 38
2	0	0	9.2	1.5 to 35
2	0	1	14	4 to 35
2	0	2	20	5 to 38
2	1	0	15	4 to 38
2	1	1	20	5 to 38
2	1	2	27	9 to 94
2	2	0	21	5 to 40
2	2	1	28	9 to 94
2	2	2	35	9 to 94
2	3	0	29	9 to 94
2	3	1	36	9 to 94
3	0	0	23	5 to 94
3	0	1	38	9 to 104
3	0	2	64	16 to 181
3	1	0	43	9 to 181
3	1	1	75	17 to 199
3	1	2	120	30 to 360
3	2	0	93	18 to 360
3	2	1	150	30 to 380
3	2	2	210	30 to 400
3	2	3	290	90 to 990
3	3	0	240	40 to 990
3	3	1	460	90 to 1980
3	3	2	1100	200 to 4000
3	3	3	> 1100	

4.6. Results and interpretation

When verifying the suitability of the membrane filtration method or the spread-plate method, a mean count of all test bacteria should be within 1/2 - 2 times the measured value of the control solution (not including the product) defined in 4.5.2. When verifying the suitability of the MPN method, the calculated value must be within the 95% confidence interval of the results obtained from the control solution. If the above criteria cannot be met in one or more of the tested strains when using any of the above methods, test products using the methods and test conditions that are the closest to the criteria.

5. Testing of Products

5.1. Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the collected sample, as described above. For fluids or solids in aerosol form, take 10 sample containers. For transdermal patches, take 10 sample patches. The tested amount may be reduced if the amount of active pharmaceutical ingredients is not greater than 1 mg per dosage unit (e.g., tablets, capsules, and injections) or if the amount of active ingredient (for formulations not presented in dosage units) in 1 g or 1 mL is less than 1 mg. In these cases, the amount used for the test to be collected should not be less than the amount contained in 10 dosage units or 10 g or 10 mL of the product. For substances used as active pharmaceutical ingredients, if the amount of a sample is limited and the batch size is very small (e.g., less than 1000 mL or 1000 g), the tested amount shall be 1% of the batch, as long as a lesser amount is specified or there are no other justifiable reasons. For samples where the batch size is less than 200 (e.g., investigational samples), the tested amount may be reduced to 2 units or 1 unit (if the batch size is less than 100). Randomly collect the samples from the container of the raw material or formulation. To obtain the required quantity of the samples, mix the contents of a sufficient number of containers.

5.2. Product test

5.2.1. Membrane filtration method

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare a sample using a method for which suitability has been confirmed in the presence of the product as described in 4.5., transfer an appropriate amount to 2 membrane filters respectively, and filter it immediately. Clean each filter using a method for which suitability has been confirmed. For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean-Casein Digest Agar, and another membrane filter to the surface of a Sabouraud Dextrose Agar for TYMC. Incubate the plate of Soybean-Casein Digest Agar at 30

- 35 °C for 3 - 5 days and the plate of Sabouraud Dextrose Agar at 20 - 25 °C for 5 - 7 days. Calculate the number of CFU per 1 g or 1 mL of the sample. When examining transdermal patches, filter 10% of the volume of the preparation described under 4.5.1 separately through 2 sterile membrane filters. Transfer one membrane to Soybean-Casein Digest Agar for TAMC and the other membrane to Sabouraud Dextrose Agar for TYMC.

5.2.2. Plate-count methods

5.2.2.1. Pour-plate method

Prepare the test solution using a method for which suitability has been confirmed as per (4). Prepare at least 2 Petri dishes for each medium at each level of dilution. Incubate the Soybean-Casein Digest Agar at 30 - 35 °C for 3 - 5 days and the Sabouraud Dextrose Agar at 20 - 25 °C for 5 - 7 days. Select the agar medium with the dilution showing the largest number of colonies, less than 250 for TAMC and 50 for TYMC. Calculate the colony count per 1 g or 1 mL of the sample by calculating the arithmetic mean of the counts in each medium.

5.2.2.2. Spread plate method

Prepare the test solution using a method for which suitability has been confirmed as per (4). Prepare at least 2 Petri dishes for each medium at each level of dilution. Incubation and the calculation of colony count is performed as described for the pour-plate method.

5.2.3. Most-probable-number method

Prepare and dilute the test solution using a method that has been confirmed as described in section (4) of 4. Incubate all test tubes at 30 - 35 °C for 3 - 5 days. If necessary, subculture using a procedure for which suitability has been confirmed. Record the number of test tubes showing microbial growth at each level of dilution. Determine the MPN of microorganisms per 1 g or 1 mL of the product from Table I-3.

5.3. Interpretation of the results

The colony count found using Soybean-Casein Digest Agar Medium is the total aerobic microbial count (TAMC). The fungal colony count on this medium is considered to be TAMC. The total combined yeasts/molds count (TYMC) using a Sabouraud Dextrose Agar is considered to be equal to the number of the total combined yeasts/molds count(TYMC). If colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, Sabouraud Dextrose Agar containing antibiotics may be used.

If the MPN method is used for counting, the calculated value is considered to be the TAMC. When microbiological quality acceptance criteria are specified, results are interpreted as follows:

- 10^1 CFU: maximum acceptable count = 20,
- 10^2 CFU: maximum acceptable count = 200,
- 10^3 CFU: maximum acceptable count = 2000, and so forth.

The recommended solutions and media are specified in “Tests for specified microorganisms.”

II. Microbiological Test of Non-Sterile Products: Tests for specified microorganisms

1. Introduction

The tests described in the following will allow determination of the absence, or limited occurrence of specified microorganisms which may be detected under the conditions described. The primary purpose of the test is to determine whether a substance or formulation complies with an established specification for microbiological quality. Follow the suggested instructions and interpret the results, including the number of collected sample. Alternative microbiological procedures, including automated methods may be used, provided that they have equivalence to the test specified in this chapter.

2. General Procedures

The samples are prepared as described in “Total Microbial Count.” If the sample has antimicrobial activity, it should be removed or neutralized as much as possible as described in “Total Microbial Count.” If surface-active substances are used for sample preparation, make sure that they have no toxicity for microorganisms and interaction with inactivators as described in “Total Microbial Count."

3. Growth Promoting, Suitability of the Test and Negative Controls

The ability of the test to detect microorganisms in the presence of products to be tested must be confirmed. Then, confirm the suitability when there are changes to the test or product that may affect the results of the test.

3.1. Preparation of test strains

Prepare test bacteria using standardized stable suspensions or by the following method. Manage microorganisms used for testing with a seed-lot system to ensure that they are NMT 5 passages removed from the original master seed-lot.

3.1.1. Aerobic microorganisms

Incubate the bacterial test strains in containers containing Soybean-Casein Digest Broth or on Soybean-Casein Digest Agar at 30 to 35 ℃ for 18 to 24 hours, respectively. Separately , incubate the test strain for Candida albicans on Sabouraud Dextrose Agar or in Sabouraud Dextrose Broth at 20 to 25 ℃ for 2 to 3 days, respectively.

Staphylococcus aureus : e.g., ATCC 6538, NCIMB 9518, CIP 4.83, NBRC 13276 or KCTC 1927

Pseudomonas aeruginosa : e.g., ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275 or KCTC 2513

Escherichia coli : e.g., ATCC 8739,
NCIMB 8545, CIP 53.126, NBRC 3972 or KCTC 2571

*Salmonella enterica*subsp. *enterica* serovar Typhimurium: e.g., ATCC 14028 or equivalent, its equivalent or *Salmonella enterica* subsp. *enterica* serovar Abony) : e.g., NBRC 100797, NCTC 6017 or CIP 80.39

Candida albicans : e.g., ATCC 10231, NCPF 3179, IP 48.72, NBRC 1594 or KCTC 7965

In preparing the suspension for test strains, use the buffered sodium chloride-peptone solution, pH 7.0 or phosphate buffer solution, pH 7.2. Use the suspensions within 2 hours, or if stored at 2 - 8 ℃, use within 24 hours.

3.1.2. Clostridia

Use Clostridia, e.g., ATCC 11437(NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404(NCTC 532 또는 CIP 79.3). Inoculate the Reinforced Medium for Clostridia with the test strain of Clostridia and incubate it at 30 - 35 °C for 24 - 48 hours under anaerobic conditions. Instead of preparing and diluting a fresh suspension of vegetative cells from *C. sporogenes*, spore suspension may be used as inoculum solution. The spore suspension may be maintained at 2 - 8 ℃ for a validated period.

3.2. Negative control

To check testing conditions, test a negative control using the chosen diluents in place of the test preparation. There must be no growth of microorganisms. A negative control is also performed when testing the products as described in 4. A failed negative control should be investigated.

Table II-1 Growth promotion and inhibition of media and indicative properties

Medium	Property	Test strains
Test for bile-tolerant gram-negative bacteria		
Enterobacteria Enrichment Broth Mossel	Growth promoting	*E. coli* *P. aeruginosa*
	a) Growth inhibiting	*S. aureus*
VRB (Violet/Red/Bile) Agar with glucose	Growth promoting + Indicative	*E. coli* *P. aeruginosa*
Test for Escherichiacoli		
MacConkey Broth	Growth promoting	*E. coli*
	b) Growth inhibiting	*S. aureus*
MacConkey Agar	Growth promoting + Indicative	*E. coli*
Test for Salmonella		
Rappaport-Vassiliadis Salmonella Enrichment Broth	Growth promoting	*Salmonella enterica subsp. enterica* serovar Typhimurium or *Salmonella enterica subsp. enterica* serovar Abony
	c) Growth inhibiting	*S. aureus*
XLD (Xylose-Lysine-Desoxycholate) Agar	Growth promoting + Indicative	*Salmonella enterica subsp. enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
Test for Pseudomonasaeruginosa		
Cetrimide Agar	Growth promoting	*P. aeruginosa*
	d) Growth inhibiting	*E. coli*
Test for Staphylococcusaureus		
Mannitol Salt Agar	Growth promoting + Indicative	*S. aureus*
	e) Growth inhibiting	*E. coli*
Test for Clostridia		
Reinforced Medium for Clostridia	Growth promoting	*Cl. sporogenes*
Columbia Agar	Growth promoting	*Cl. sporogenes*
Test for Candidaalbicans		
Sabouraud Dextrose Broth	Growth promoting	*C. albicans*
Sabouraud Dextrose Agar	Growth promoting + Indicative	*C. albicans*

3.3. Growth promotion of media

For ready-prepared media, perform tests for each batch. Test each batch of the dry medium or the medium prepared from each ingredient. Identify suitable properties of the relevant media as described in Table II-1.

Test for growth promoting properties, broth media Inoculate a small portion of the appropriate medium with a small number (NMT 100 CFU) of the appropriate microorganism. Incubate it at the specified temperature within the shortest period of time specified in the test. Make sure that the growth of microorganism is equivalent to that previously obtained in the same type of medium where efficacy has been confirmed.

Test for growth promoting properties, solid media Inoculate each plate with a small number (NMT 100 CFU) of the appropriate microorganisms and perform the spread plate method. Incubate it at the specified temperature for less than the shortest period of time specified in the test. Make sure that the growth of microorganisms is equivalent to that previously obtained in the same type of medium where efficacy has been confirmed.

Test for inhibitory properties, broth or solid media Inoculate the appropriate medium to at least 100 CFU of the appropriate microorganism. Incubate it at the specified temperature for NLT the longest period of time specified in the test. No growth of the test bacteria is identified.

Test for indicative properties Inoculate each plate with a small number (NMT 100 CFU) of the appropriate microorganisms and perform tests using the spread plate method. Incubate at the specified temperature, and the incubation time should be within the incubation period specified in the test. Make sure that the appearance and indication reactions of the colonies are equivalent to that previously obtained in the same type of medium from the batches where efficacy has been confirmed.

3.4. Suitability of the test

For each sample, prepare test solutions as described in the relevant paragraphs in 4. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use microorganisms with a number of bacteria NMT 100 CFU in the inoculated test solution. Perform the test as described in the relevant paragraphs in 4. However, apply the shortest time among the specified incubation periods. Some specified microorganisms must be detected with the indication reactions as described in 4. Any antimicrobial activity of the product necessitates a modification of the test procedure (See section 4.5.3. Neutralization and removal of antimicrobial activity in "Total Microbial Count.") In a specific product, if the antimicrobial activity of a microorganism cannot be neutralized with the specified test, then it is assumed that the inhibited microorganism is not present in the sample.

4. Product test

4.1. Bile-tolerant gram-negative bacteria

4.1.1. Sample preparation and pre-incubation

Prepare a 1 in 10 diluent of no less than 1 g of sample as described in "Total Microbial Count," but at this time using soybean casein digest broth as the chosen diluents, mix and incubate at 20 – 25 °C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours, within 5 hours).

4.1.2. Test for absence

Unless otherwise specified, inoculate the test solution equivalent to 1 g of sample, prepared in 4.1.1., to Enterobacteria enrichment broth Mossel medium.

After incubating at 30 – 35 °C for 24 – 48 hours, subculture the sample on plates of VRBG (Violet Red Bile Glucose) Agar and incubate at 30 – 35 °C for 18 – 24 hours. The product is considered to be suitable for the test if there is no growth of colonies.

4.1.3. Assay

4.1.3.1. Selection and subculture

Inoculate 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of sample to the appropriate amount of Enterobacteria enrichment broth Mossel respectively, by using the preparation solution and/or diluted solution described in 4.1.1. After

incubating at 30 – 35 °C for 24 – 48 hours, subculture the sample on plates of VRBG (Violet Red Bile Glucose) Agar and incubate at 30 – 35 °C for 18 – 24 hours.

4.1.3.2 Interpretation

If growth of colonies is approved, interpret as positive. Specify the smallest quantity of the product that gives a positive result and the largest quantity of the product that gives a negative result. Calculate the probable number of bacteria from Table II-2.

Table II-2. Interpretation of results

Results for each quantity of product			Probable number of bacteria per 1 g or 1 mL of the product
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	NLT 10^3
+	+	-	NMT 10^3 and NLT 10^2
+	-	-	NMT 10^2 and NLT10
-	-	-	less than 10

4.2. Escherichia coli

4.2.1. Sample preparation and pre-incubation

Prepare a 1 in 10 diluent of NLT 1 g of sample as described in "Total Microbial Count". Incubate this solution of 10 mL or the amount equivalent to 1 g or 1 mL of sample to a suitable amount (determined as described in 3.4) of soybean casein digest broth. Mix and incubate at 30 – 35 °C for 18 – 24 hours.

4.2.2. Selection and subculture

After shaking the container with culture medium, previously cultured, add 1 mL of soybean casein digest broth to 100 mL of MacConkey broth medium. Incubate at 42 -44 °C for 24 - 48 hours. Subculture on Fluid MacConky Broth and incubate at 30 - 35 °C for 18 - 72 hours.

4.2.3. Interpretation

Growth of colonies indicates the possible presence of Escherichia coli. At this time, it may be suspected as positive, and confirmed by the Identification. The sample is suitable for the test if no colonies are present or if the Identification is negative.

4.3. Salmonella

4.3.1. Sample preparation and pre-incubation

Prepare a sample according to the Total Microbial Count, incubate the amount equivalent to more than 10 g or 10 mL of sample to a suitable amount (determined as described in 3.4) of soybean casein digest broth. Mix and culture at 30 – 35 °C for 18 – 24 hours.

4.3.2. Selection and subculture

Inoculate 0.1 mL of Soybean Casein Digest Broth to 10 mL of Rappaport-Vassiliadis Salmonella Enrichment Broth. Incubate at 30 – 35 °C for 18 – 24 hours, and subculture on plates of XLD (Xylose-Lysine-Desoxy-cholate) agar. Incubate at 30 – 35 °C for 18 – 48 hours.

4.3.3. Interpretation

The possible presence of Salmonella is indicated by the growth of well-developed, red colonies, with or without a black center. At this time, it may be suspected as positive, and confirmed by the Identification. The product is suitable with the test if colonies of the types described are not present or if the confirmatory identification test is negative.

4.4. Pseudomonas aeruginosa

4.4.1. Sample preparation and pre-incubation

Prepare a 1 in 10 diluent of NLT 1 g of sample as described in "Total Microbial Count." Incubate this solution of 10 mL or the amount equivalent to 1 g or 1 mL of sample to a suitable amount (determined as described in 3.4) of soybean casein digest broth, and mix. When testing transdermal patches, prepare sample as described in 4.5.1 of "Total Microbial Count," filter the volume of sample corresponding to 1 patch through a sterile membrane filter, and place this filter in 100 mL of Soybean-Casein Digest Broth. Incubate at 30 to 35 °C for 18 to 24 hours.

4.4.2. Selection and subculture

Subculture on a plate of Cetrimide agar medium and incubate at 30 to 35 °C for 18 to 72 hours.

4.4.3. Interpretation

Colony growth indicates the possible presence of Pseudomonas aeruginosa. At this time, it may be suspected as positive, and confirmed by the Identification. The product is suitable for the test if no colonies are present or if the Identification is negative.

4.5. Staphylococcus aureus

4.5.1. Sample preparation and pre-incubation

Prepare a 1 in 10 diluent of NLT 1 g of sample as described in "Total Microbial Count". Incubate this solution of 10 mL or the amount equivalent to 1 g or 1 mL of sample to a suitable amount (determined as described in 3.4) of Soybean-Casein Digest Broth, and mix. Incubate at 30 to 35 °C for 18 – 24 hours. When testing transdermal patches, prepare a sample as described in 4.5.1 of "Total Microbial Count," filter the volume of sample corresponding to 1 patch through a sterile membrane filter, and place this filter in 100 mL of Soybean-Casein Digest Agar.

4.5.2. Selection and subculture

Subculture on a plate of Mannitol sodium chloride agar medium and incubate at 30 to 35 °C for 18 to 72 hours.

4.5.3. Interpretation

The growth of yellow or white colonies surrounded by a yellow band indicates the possible presence of Staphylococcus aureus. At this time, it may be suspected to be positive. This is confirmed by the Identification. The product is suitable for the test if colonies of the above type are not present or if the confirmatory identification tests are negative.

4.6. Clostridia

4.6.1. Sample preparation and heat treatment

Prepare a 1 in 10 diluent (at least 20 mL) of NLT 2 g or 2 mL of sample as described in "Total Microbial Count." Divide the sample into two portions of at least 10 mL. Heat 1 portion at 80 °C for 10 min and cool rapidly. Do not heat the other portion.

4.6.2. Selection and subculture

Inoculate 10 mL or the amount equivalent to 1 g or 1 mL of sample to a suitable amount (determined as described in 3.4) of reinforced medium for Clostridia. Incubate under anaerobic conditions at 30 – 35 °C for 48 hours. After incubation, transfer the contents of each tube to Columbia agar medium, and incubate under anaerobic conditions at 30 – 35 °C for 48 - 72 hours.

4.6.3. Interpretation

The occurrence of anaerobic growth of bacilli (with or without endospores) giving a negative catalase reaction indicates the positive presence of Clostridia. At this time, it may be suspected to be positive. This is confirmed by the Identification. This sample is suitable for the test if colonies of the above type are not present or if the confirmatory identification tests are negative.

4.7. Candida albicans

4.7.1. Sample preparation and pre-incubation

Prepare the sample as specified in the「Microbial Enumeration Test」. Inoculate 10 mL or the amount equivalent to 1 g or 1 mL of sample to 100 mL of Sabouraud dextrose broth. Incubate at 30 to 35 ℃ for 3 to 5 days.

4.7.2. Selection and subculture

Subculture on a plate of Sabouraud Dextrose Agar and incubate at 30 to 35 ℃ for 24 to 48 hours.

4.7.3. Interpretation

The growth of white colonies indicates the possible presence of Candida albicans.. At this time, it may be suspected to be positive. This is confirmed by the Identification. The product is suitable for the test if such white colonies are not present or if the Identification are negative.

The following section is given to provide additional information.

5. Recommended Solutions and Culture Media

The following solutions and media are suitable for the purpose specified in microbial test of this chapter. Other media may be used provided that the suitability can be demonstrated.

Stock Buffer Solution Dissolve 34 g of potassium dihydrogen phosphate in 500 mL of water, adjust to pH 7.0 - 7.4 with sodium hydroxide TS, and add purified water to make it up to a final volume of 1000 mL. Divide into containers and sterilize. Preserve at a temperature of 2 - 8 ℃.

Phosphate Buffer (pH 7.2) Prepare a mixture of water and stock buffer solution (800 : 1) and sterilize.

Buffered Sodium Chloride - Pepton Solution, pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate (Equivalent to 0.067 mol/L phosphate)	7.2 g
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave at validated intervals.

Soybean-Casein Digest Broth

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Adjust the pH to 7.1 – 7.5 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals.

Soybean-Casein Digest Agar

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH to 7.1 – 7.5 at 25 °C after sterilization. Sterilize the medium in an autoclave at validated intervals.

Sabouraud Dextrose Agar

Glucose	40.0 g
Peptones (meat:casein=1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH to 5.4 – 5.8 at 25 °C after sterilization.Sterilize in an autoclave at validated intervals.

Potato Dextrose Agar

Infusion from potatoes	200 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH to 5.4 – 5.8 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals.

Sabouraud Dextrose Broth

Glucose	20.0 g
Peptones (meat:casein=1:1)	10.0 g
Water	1000 mL

Adjust the pH to 5.4 – 5.8 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals.

Enterobacteria Enrichment Broth Mossel

Gelatin peptone	10.0 g
Glucose monohydrate	5.0 g
Dehydrated ox bile	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dihydrate	8.0 g
Brilliant green	15 mg
Water	1000 mL

Adjust the pH to 7.0 - 7.4 at 25 °C after sterilization. Heat the medium at 100 °C for 30 min and cool it immediately.

Violet Red Bile Glucose Agar

Yeast extract	3.0 g
Gelatin peptone	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Methylrosaniline chloride	2 mg
Water	1000 mL

Adjust the pH to 7.2 - 7.6 at 25 °C after sterilization. Heat the medium to boiling. Do not heat in an autoclave.

MacConkey Broth

Gelatin peptone	20.0 g
Lactose monohydrate	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Water	1000 mL

Adjust the pH to 7.1 – 7.5 at 25 °C after sterilization. Sterilize the medium in an autoclave at validated intervals.

MacConkey Agar

Gelatin peptone	17.0 g
Peptone (meat and casein)	3.0 g
Lactose monohydrate	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30 mg
Methylrosaniline chloride	1 mg
Water	1000 mL

Adjust the pH to 6.9 - 7.3 at 25 ℃ after sterilization. Boil the medium for 1 min, while constantly stirring, and sterilize in an autoclave at validated intervals.

Rappaport-Vassiliadis Salmonella Enrichment Broth

Soybean peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dibasic potassium phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	36 mg
Water	1000 mL

Dissolve the ingredients by slightly warming and sterilize it in an autoclave at validated intervals, at a temperature not exceeding 115 °C. After heating and autoclaving, the pH should be 5.0 - 5.4 at 25 °C.

XLD (Xylose-Lysine-Desoxycholate) Agar (XLD Agar)

Xylose	3.5 g

L-Lysine	5.0 g
Lactose monohydrate	7.5 g
White sugar	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulfate pentahydrate	6.8 g
Ammonium iron (III) citrate	0.8 g
Water	1000 mL

Adjust the pH to 7.2 - 7.6 at 25 °C after heating. Heat the medium to boiling, cool to 50 °C, and pour into a Petri dish. Do not heat in an autoclave.

Cetrimide Agar

Gelatin peptone	20.0 g
Magnesium chloride hexahydrate	1.4 g
Potassium sulfate	10.0 g
Cetrimid	0.3 g
Agar	13.6 g
Water	1000 mL
Glycine	10.0 mL

Heat to boiling for 1 min while shaking. Adjust the pH to 7.0 – 7.4 at 25 °C after sterilization. Sterilize in an autoclave using a validated cycle. Sterilize in an autoclave at validated intervals.

Mannitol Salt Agar

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Water	1000 mL

Heat to boiling for 1 min while shaking. Adjust the pH to 7.2 - 7.6 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals.

Reinforced Medium for Clostridia

Meat extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose monohydrate	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	0.5 g
Water	1000 mL

Hydrate the agar, dissolve by heating to boiling while stirring continuously. If necessary, adjust the pH to 6.6 - 7.0 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals.

Columbia Agar

Casein peptone	10.0 g
Meat peptic digest	5.0 g
Heart pancreatic digest	3.0 g
Yeast extract	5.0 g
Corn starch	1.0 g
Sodium chloride	5.0 g
Agar (according to gelling power)	10.0 g to 15.0 g
Water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH to 7.1 - 7.5 at 25 °C after sterilization. Sterilize in an autoclave at validated intervals. Cool it to 45 - 50 °C. If necessary, add gentamicin sulfate corresponding to 20 mg of gentamicin base to the medium made of the above composition and pour into a Petri dish.

III. Microbiological quality characteristics of non-sterile pharmaceutical products

In non-sterile products, the presence of certain microorganisms can reduce or inactivate the medicinal activity of the product, and may also have a negative impact on the patient's health. For this reason, pharmaceutical manufacturers must comply with the latest guidelines when manufacturing, storing, and distributing products and conduct quality control to minimize biological hazards in the final products. Microbial testing of non-sterile products follows the Total Microbial Count and Tests for Specified Microorganisms under the Microbiological Examination of Non-sterile Products in this chapter.

The acceptance criteria for total aerobic microorganisms (TAMC) and total combined yeasts/mould count (TYMC) for non-sterile products are shown in Table III-1. Microbiological acceptance criteria are determined by each test result or repeated measurement value in the case of repeated measurements. When microbiological quality acceptance criteria are specified, they are judged as follows:

- 10^1 CFU: maximum acceptable count = 20,
- 10^2 CFU: maximum acceptable count = 200,
- 10^3 CFU: maximum acceptable count = 2000,

Table III-1 also shows specific microorganisms that should not be present in preparations for which microbial acceptance criteria have been established. However, since this table does not describe all specific microorganisms that should not be detected, testing for other microorganisms is also required depending on the characteristics of the starting material or manufacturing process for a specific product.

Table III-1. Acceptance criteria for microbiological quality of non-sterile products

Administration route	Total aerobic microbial count (CFU/g or CFU/mL)	Total combined yeast/mould count (CFU/g or CFU/mL)	Specific Microorganism
Oral non-aqueous preparation	1 × 103	1 × 102	Escherichia coli not detected (1 g or 1 mL)
Oral aqueous preparation	1 × 102	1 × 101	Escherichia coli not detected (1 g or 1 mL)
Preparations applied to the rectum	1 × 103	1 × 102	-
Preparations applied to the oral cavity (including gingival preparations) Preparations applied to the skin Preparations administered to the ear Preparations applied to the	1 × 102	1 × 101	Staphylococcus aureus and Pseudomonas aeruginosa not detected (1 g or 1 mL)

Administration route	Total aerobic microbial count (CFU/g or CFU/mL)	Total combined yeast/mould count (CFU/g or CFU/mL)	Specific Microorganism
nose			
Preparations applied to the vagina	1 × 102	1 × 101	Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans not detected (1 g or 1 mL)
Transdermal patches (limit per patch including adhesive layer and support)	1 × 102	1 × 101	Staphylococcus aureus and Pseudomonas aeruginosa not detected (1 patch)
Inhalations (Special requirements apply for inhalation solution for nebulization)	1 × 102	1 × 101	Staphylococcus aureus, Pseudomonas aeruginosa, bile-tolerant gram-negative bacteria not detected (1 g or 1 mL)

If it is proven that the prescribed test cannot effectively measure the specified level of microorganisms, a validated test with a detection limit as close as possible to the proposed acceptance criteria can be used.

In addition to the microorganisms listed in Table III-1, the importance of other recovered microorganisms is evaluated as follows:

Product use: Risk factors depend on the route of administration (eyes, nose, respiratory tract).

Characteristics of the product: Does the product support the growth of microorganisms or does it have sufficient antibacterial activity?

How to apply:

User: The level of risk varies for newborns, infants, and debilitated people.

Immune response suppressor: use of corticosteroids

Presence of disease, trauma or organ damage

Risk assessment of relevant factors is performed by staff who have been specially trained in microbiology and the interpretation of microbiological data.

Factors to consider when assessing the risk of raw materials include the process through which the raw material is used in the product, the latest testing technology, and the availability of raw materials of the desired quality.

Table III-2. Microbial limit standards for non-sterile raw materials of pharmaceuticals

	Total aerobic microbial count (CFU/g or CFU/mL)	Total yeast/mould count (CFU/g or CFU/mL)
Pharmaceutical raw materials	1×10^3	1×10^2

Table IV-1 Scope and standards for crude drugs, etc. under the Microbiological Examination of Non-sterile Products

Scope of application, etc.	Limit criteria (colony/1 g or 1 mL)		
	Total aerobic microbial count[1)]	Total combined yeasts/mould count	Specific microorganism[2)]
Herbal medicine extract Oral solids containing herbal medicine extract	NMT 1×10^5	NMT 1×10^2	Undetected
Oral solids containing NLT 1 type of unextracted herbal medicine Oral solids in a mixture of herbal medicine extract and unextracted herbal medicine	-	-	Undetected
Oral liquid (herbal medicine)	NMT 1×10^3	NMT 1×10^2	Undetected
Contact lens care products	NMT 1×10^2	10 .	Undetected

1) Probiotics are excluded from bacterial testing.
2) Specific microorganisms: Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus

Mid-infrared Spectroscopy
적외부스펙트럼측정법

The Mid-infrared Spectrophotometry is a method used to measure the level of absorption or the transmittance of infrared radiation on each wavenumber when it passes through the sample. In the graphic representation of the infrared spectrum, the plot usually shows units of wavenumbers as the abscissa and units of transmittance or absorbance as the ordinate. Wavenumbers and transmittance (or absorbance) at each absorption peak may be obtained from a graph or through a calculation from the data processor. Since the wavenumber and the respective intensity of an infrared spectrum depend on the chemical structure of a substance, they can be used for the identification or quantification of a substance.

Apparatus and adjustment

Dispersive Infrared Spectrophotometer or Fourier Transform Infrared Spectrophotometer is used. Adjust the spectrophotometers in advance, and ensure that the resolution and reproducibility of transmittance or wavenumber comply with the following conditions: When measuring the absorption spectrum of a polystyrene film with a thickness of about 0.04 mm, the difference between the minimum transmittance (%) around 2870 cm^{-1} and the maximum transmittance (%) around 2850 cm^{-1} must be no less than 18%. Also, the difference between the minimum transmittance (%) around 1589 cm^{-1} and the maximum transmittance (%) around 1583 cm^{-1}must be at least 12%. The wavenumber scale is usually calibrated using some of absorption wavenumbers (cm^{-1}) under a polystyrene film. Also, the numbers in parentheses indicate the tolerance of these values.

3060.0 (±1.5) 2849.5 (±1.5) 1942.9 (±1.5)
1601.2 (±1.0) 1583.0 (±1.0) 1154.5 (±1.0)
1028.3 (±1.0)

However, when the dispersion apparatus is used, the tolerance should be within 1601.2 ± 2.0 cm^{-1} at 1601.2 cm^{-1} and within 1028.3 ± 2.0 cm^{-1} at 1028.3 cm^{-1}. For the reproducibility of transmittance and wavenumber, the difference in transmittances should be within 0.5% when measuring the transmittances of a polystyrene film twice at different wave numbers from 3000 to 1000 cm^{-1}, and the difference in wavenumbers should be within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1}.

Preparation of sample and measurement

Unless otherwise specified, when it is specified in the monograph that the test is to be performed after drying the sample, use a sample dried under the conditions specified in the Loss on drying' section. Prepare the sample using one of the following procedures to ensure that the transmittance of most absorption bands falls into the range of 5 - 80%. For the optical plate, sodium chloride, potassium bromide, etc., are used. Generally, when a double-beam-type device is used, the control is placed on the side of the reference beam. In contrast, when a single-beam-type device is used, it is placed in the same optical path as the sample and measured separately under the same operating conditions. The method of deciding the control varies depending on the sample preparation method, and sometimes the background absorption of the atmosphere can be utilized. Unless otherwise specified in the monograph, the absorption spectrum of the sample is usually measured at the wavenumber between 4000 and 400 cm-1. Measurement of the absorption spectrum is performed under the same operating conditions used for measurement of the resolving power and the precision of wave number scale and wave numbers. In this case, if the infrared spectrum of the reference standard acquired from 1) Potassium bromide disk or potassium chloride disk method, 2) Solution method, and 3) Paste method shows similar results when compared to the infrared spectrum of 7) ATR method, 7) ATR method can be applied for the Identification.

1) Potassium bromide disk or potassium chloride disk method

Powder 1 to 2 mg of a solid sample in an agate mortar, add 0.10 to 0.20 g of potassium bromide or potassium chloride used for infrared spectrophotometry with precautions against moisture absorption, triturate immediately and mix them well, and compress the mixture with a press in a suitable die (disk-forming container) to prepare the sample disk. Generally, a potassium bromide reference disk or a potassium chloride reference disk are prepared in the same manner. However, if necessary, press the mixture under reduced pressure within 0.67 kPa, and apply pressure of 50 to 100 kN (5000 - 10000 kg) per unit area (cm^2) for 5 - 8 minutes to prepare a transparent disk.

2) Solution method

Place a test solution prepared using the method specified in the monograph in a fixed cell for liquid and measure the spectrum. The solvent used for preparing the sample is usually used as the blank. The solvent used in this method should not have any interaction or chemical reaction with the sample or damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

3) Paste method

Powder 5 to 10 mg of a solid sample in an agate mortar, and, unless otherwise specified, mix with 1 or 2 drops of liquid paraffin to prepare a sample paste. After thinly spreading the paste in the center of an optical plate, place it between another optical plate with precautions against air intrusion, and measure its absorption spectrum.

4) Liquid film method

Perform measurement by placing 1 to 2 drops of a liquid sample as a thin film held between two optical plates. If it is necessary to thicken the liquid film, place a spacer, such as aluminum foil, etc., between two optical plates so the liquid sample is in the gap.

5) Film method

Perform measurement after using a sample as a thin film as it is or preparing a thin film with the sample as directed in the monograph.

6) Gas sampling method

Introduce a sample gas to a gas cell previously evacuated under the pressure specified in the monograph and measure its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm.If necessary, a cell with an optical path of 1 m or longer can be used.

7) ATR method

Place a sample closer to the side of the attenuated total reflectance (ATR) prism and examine its reflectance spectrum.

8) Diffuse reflectance method

Powder l to 3 mg of a solid sample into a fine powder of NMT about 50 μm in particle size in an agate mortar, add 0.05 to 0.10 g of potassium bromide or potassium chloride used to infrared spectrophotometry, and mix them immediately with precautions against moisture absorption. Place the mixture in a sample cup, and measure its reflectance spectrum.

Identification

1) Identification through the use of reference standard

When the spectrum of a sample and the reference standard exhibit similar absorption intensities at the same wavenumber, it can be determined that the sample and reference standard are the same materials. If the treatment method when the spectrum of the solid sample differs from the reference standard specified in the monograph, treat the sample and the reference standard under the same conditions and repeat the measurement.

2) Identification using absorption wave number

When the absorption wavenumber of the material to be identified is the same as the one specified in the monograph, it can be determined that the sample is the same material as the drug in the monograph.

Mineral Oil
광유시험법

The Mineral Oil is performed to test for mineral oil in the non-aqueous solvents used in injections and ophthalmic solutions.

Procedure

Put 10 mL of the sample into a 100 mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol. Place a small funnel with short leg on the mouth of the flask, legs side down, and heat in a water–bath until it is clear, shaking it up sometimes. Then, transfer it to a shallow porcelain dish and heat on a water bath to evaporate ethanol. Add 100 mL of water to the residue so that the Solution is not turbid when heated on a water bath.

Minimum Fill
질량·용량시험법

1. Preparations with a labeled amount of 150 g (or 150 mL) or less

A. Gels, granules, lotions, powders, syrups, liquids and solutions, ointments, emulsions, ophthalmic solutions, creams and suspensions, etc.

However, preparations for cutaneous application are included, and preparations in unit-dose packages are excluded.

1) Preparations indicated in the unit of mass

Acceptance criteria This test meets the following acceptance criteria. The average net content of the 10 containers is NLT the labeled amount, and the net content of any single container is ≥ 90% of the labeled amount where the labeled amount is ≤ 60 g, or ≥ 95% of the labeled amount where the labeled amount is > 60 g and ≤ 150 g. If these criteria are not met, determine the content of 20 additional containers. The average weight of the 30 containers must be no less than the labeled amount, and the net content of NMT 1 of the 30 containers is ≤ 90% of the labeled amount where the labeled amount is ≤ 60 g, or ≤ 95% of the labeled amount where the labeled amount is > 60 g and ≤ 150 g.

Test Method Select a sample of 10 filled containers, thoroughly cleanse, dry the outside of the containers by a suitable means, and weigh accurately each weight. At this time, assign numbers to each container for identification and make sure that each preparation corresponds to its weight. Quantitatively remove the contents from each container, rinse with a suitable solvent, dry, and weigh accurately each empty container (together with its corresponding parts at initial weight measurement, if any). Determine the net weight of the contents for each container by subtracting the weight of the empty container from the weight of the corresponding content.

2) Preparations indicated in the unit of volume

Acceptance criteria This test meets the following acceptance criteria. The average volume of the 10 containers is NLT the labeled amount, and the net content of any single container is ≥ 90% of the labeled amount where the labeled amount is ≤ 60 mL, or ≥ 95% of the labeled amount where the labeled amount is > 60 mL and ≤ 150 mL. If these criteria are not met, determine the content of 20 additional containers. The average volume of the 30 containers must be no less than the labeled amount, and the net content of NMT 1 of the 30 containers ≤ 90% of the labeled amount where the labeled amount is ≤ 60 mL, or ≤ 95% of the labeled amount where the labeled amount is > 60 mL and ≤ 150 mL.

Test method Select 10 preparations and measure the volume of each preparation directly with a measuring cylinder. Alternatively, add water into the container with the contents using a burette and precisely measure the amount of water used to fill up the container. At this point, label each container with numbers for identification, ensuring that each preparation corresponds to its volume. After completely emptying the containers, wash the inside of the containers with water or an appropriate organic solvent. Dry them, then add water again with a burette to fill up the container, and precisely measure the amount of water used. Determine the net volume of the container's content by subtracting the amount of water used to fill the container with the contents from the amount of water used to fill the container without the contents.

B. Preparations in unit-dose packages in the form of gels, liquids and solutions, emulsions or suspensions among those intended for cutaneous application

Acceptance criteria When calculating the average mass (volume) of 20 preparations, if the number of preparations for which the individual mass (volume) deviates by > 10% from the average mass (volume) is ≤ 2, and there are no preparations for which the individual mass (volume) deviates by > 25% from the average mass (volume), then such a preparation is considered to meet the requirements.

Test method Select 20 preparations and precisely measure the mass (volume) of each preparation according to the method described in 1. A.1) or 1.A.2) to determine the mass (volume) of each preparation.

C. Pills

1) Products with labeled amount of 1.5 g or more

Acceptance criteria This test meets the following acceptance criteria. When calculating the average mass of 10 preparations, if the number of preparations for which the individual mass deviates from the average mass by the specified value is ≤ 2, and there are no preparations for which the individual mass deviates from the average mass by double the specified value, then such a preparation is considered to meet the requirements.

Mean mass (g)		Variation (%)
≥ 1.5 g	< 3.0 g	8
≥ 3.0 g	< 6.0 g	7
≥ 6.0 g	< 9.0 g	6
≥ 9.0 g		5

Test method Open the packages of 10 preparations and measure the mass of each preparation.

2) Preparations in unit-dose packages

Acceptance criteria When calculating the average mass of 20 sachets, if the number of sachets with an individual mass deviation exceeding 10% from the average mass is ≤ 2, and there are no sachets with an individual mass deviation > 25% from the average mass, then the preparation is considered to meet the requirements.

Test Method Select 20 sachets of the preparation and precisely measure the mass of the content individually. At this point, label each preparation with a number for identification, ensuring each preparation corresponds to its mass. Open the sachet, remove the content, and reweigh each empty sachet accurately. Determine the net weight of the contents by difference.

2. Preparations with a labeled amount exceeding 150 g (or 150 mL)

Acceptance criteria The requirements are met if the net weight(volume) of the contents of each preparation is not less than the labeled amount.

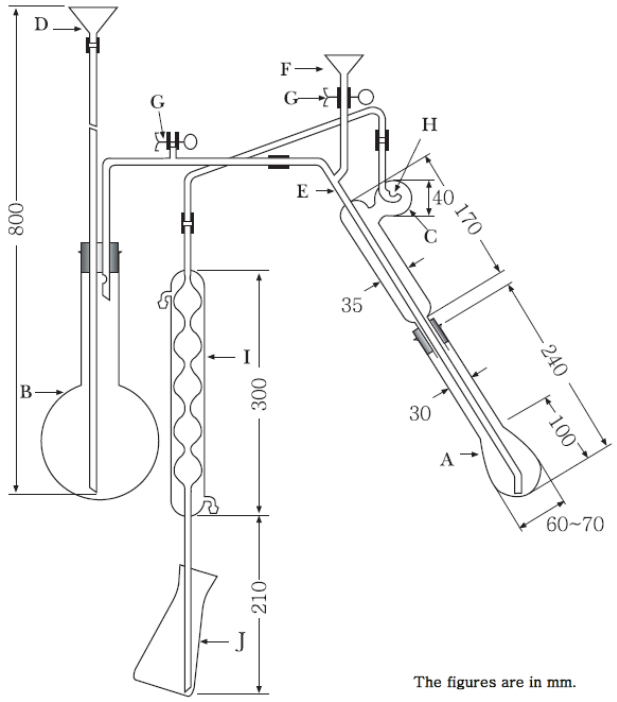
Test method Select three preparations, and directly measure their mass or volume with a measuring cylinder.

Nitrogen Determination (Semimicro-Kjeldahl Method) 질소정량법 (세미마이크로킬달법)

The Nitrogen Determination involves heating and decomposing organic compounds containing nitrogen with sulfuric acid, converting nitrogen into ammonia nitrogen, adding alkali to liberate it and quantifying the collected ammonia according to the steam distillation method.

Apparatus

Use the apparatus shown in the Figure. The entire body is made of hard glass, and ground glass surfaces may be used for joints. The rubber parts used in this apparatus are boiled in sodium hydroxide TS for 10 to 30 minutes, boiled in water for 30 to 60 minutes, and rinsed well with water before use. However, automated apparatuses in titration end detection methods (e.g., potentiometric titration and colorimetric titration) can decompose organic matter and identify the produced ammonia type and its quantification.



Figure

A: Kjeldahl flask
B: Steam generator. Add water with 2-3 drops of sulfuric acid and a boiling stone.
C: Part to prevent contents from popping up
D: Water funnel
E: Steam pipe
F: Alkaline solution funnel
G: Rubber tube with pinch cock
H: Small hole (The diameter is almost equal to the internal diameter of the pipe.)
I: Cooler (The lower end is cut off at an angle.)
J: Collector

Apparatus suitability

When using an automated apparatus, it is necessary to periodically check the suitability using the following method. Dry amidosulfuric acid (standard reagent) in a desiccator (reduced pressure, silica gel) for about 48 hours. Weigh about 1.7 g of it accurately and dissolve it in water to make exactly 200 mL. Take exactly 2 mL of this solution, put it into a decomposition flask, and operate it according to the instructions for each device below. The nitrogen content (%) in amidosulfuric acid should be within the range of 14.2 - 14.6%.

Reagent and test solution

Decomposition accelerator: Unless otherwise specified, mix 10 g of potassium sulfate and 1 g of copper (II) sulfate pentahydrate, grind it into powder, and use 1 g. The type and amount of the degradation accelerator can be changed in addition to verification using samples that show results equivalent to those specified.

Procedure

Unless otherwise specified, follow the method below. Weigh accurately or use a pipette to take an amount of sample equivalent to 2 to 3 mg of nitrogen (N: 14.01), put it into Kjeldahl flask A, add a decomposition accelerator and wash the sample on the flask neck with a small amount of water, and then add 7 mL of sulfuric acid along the inside wall of the flask. Next, carefully add 1 mL of hydrogen peroxide (30) along the inside wall while shaking the flask. Heat the sulfuric acid in the flask neck again until it liquefies in the flask neck. Stop heating when the color of the liquid changes to a bright clear green through clear blue and no carbide is visible on the inner wall of the flask. Cool it if necessary, then add a small amount of hydrogen peroxide (30) and heat it again. After cooling, carefully add 20 mL of water and cool it. Next, connect the flask to a distillation apparatus that was washed with steam in advance. Put 15 mL of boric acid solution (1 in 25) and 3 drops of bromocresol green-methyl red TS in the collector J, add an appropriate amount of water, and dip the lower end of the cooler I into this solution. Add 30 mL of sodium hydroxide solution (2 in 5) from funnel F, carefully wash it with 10 mL of water, immediately close the pinch cock of rubber tube G with a pinch cock, pass water vapor, and distill until 80 to 100 mL of emulsion is obtained. Lift the lower end of the cooler I from the surface of the liquid, rinse the immersed part with a small amount of water, and titrate with 0.005 mol/L sulfuric acid. The endpoint of the titration is when the green solution change to light grayish purple through light grayish blue. Perform correction through a blank test in the same manner.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.1401 mg of N

Nuclear Magnetic Resonance Spectroscopy 핵자기공명스펙트럼측정법

The Nuclear Magnetic Resonance (NMR) Spectroscopy is a spectral measurement method using the phenomenon that the constituent atomic nuclei of a material placed in a static magnetic field resonate with radio waves of their natural frequency and absorb radio waves as they transition from the lower to the higher energy nuclear spin state.

Nuclei to be measured are mainly ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P, etc.

The nuclear spin I of an atomic nucleus is 0, 1/2, 1, 3/2, …, n/2 (where n is an integer), etc. (I=1/2 in ^{1}H and ^{13}C). When a

nucleus is placed in a magnetic field, the nuclear moments are oriented in 2 I + 1 (2 for ^{1}H and ^{13}C) directions according to the magnetic quantum number m_I. To make a transition between aligned energy levels, a radio wave of frequency v in the following equation is required. In other words, if a nucleus with a gyromagnetic ratio of γ is placed in an external magnetic field H0, the following equation is established:

$$v = \gamma \cdot \frac{H_0}{2\pi}$$

γ: Gyromagnetic ratio
H_0: External magnetic field

Therefore, when a radio wave of frequency of v is irradiated, the resonance condition is satisfied and absorption of radio waves (NMR signal) of that frequency is observed. Since the absorption coefficient (transition probability) is constant for nuclei in any environment, the obtained NMR signal intensity is basically proportional to the number of resonant nuclei. Nuclear spins biased towards high energy levels by this transition return to the thermal equilibrium distribution after a certain period of time (relaxation), which is referred to as relaxation time.

When a molecule is placed in a magnetic field, the electrons in the molecule shield the nucleus from the external magnetic field. If the environment of the nucleus in the molecule is different, the degree of shielding the external magnetic field is different. Accordingly, each nucleus has a different resonance frequency and the different signals are observed. The position of this signal is indicated by the chemical shift δ. Since the resonance frequency changes in proportion to the magnetic field, the definition of chemical shift, which is independent of the magnetic field, is as follows:

$$\delta = \frac{\nu_S - \nu_R}{\nu_R} + \delta_R$$

ν_S: Resonance frequency of sample nucleus
ν_R: Resonance frequency of the reference nucleus
δ_R: Chemical shift of the reference nucleus (if not 0)

Chemical shift is usually expressed in the unit of ppm by setting the position of signal of reference material (reference nucleus) to 0, but if the signal position of reference material is not 0, it is calibrated using the chemical shift of pre-determined reference material.

Since the magnetic field at each nucleus in a molecule is affected not only by contributions from ambient electrons (nuclear shielding), but also by other nuclear magnets in the molecule (a nucleus with a nuclear spin is itself a magnet), the signal is split due to coupling by chemical bonds between nuclear magnets. The gap between these splits is called the spin-spin coupling constant *J*. *J* is expressed in hertz (Hz). *J* does not depend on the strength of the external magnetic field, and the pattern of split becomes complicated as the number of interacting nuclei increases.

In the NMR spectrum, basically 4 parameters, chemical shift, spin-spin coupling constant, integrated area intensity of the signal(proportional to the number in ^{1}H nuclei, but in ^{13}C nuclei, is affected by nuclear Overhauser effect (NOE), relaxation, etc.), and relaxation time are obtained. These parameters can be used for structural analysis, identification or assay. For structural analysis, various techniques such as decoupling, NOE, two-dimensional NMR, etc. can be used.

Apparatus

Spectrum is measured using a continuous wave nuclear magnetic resonance (CW-NMR) spectrum determination apparatus or a pulsed Fourier transform nuclear magnetic resonance (FT-NMR) spectrum determination apparatus.

Procedure

Adjust the sensitivity and resolution of the apparatus to the optimal conditions using ethylbenzene, 1,2-dichlorobenzene or acetaldehyde in deuterated solvent for NMR spectroscopy, and measure the spectrum by the following method:

1) Prepare the specimen tube using either the internal standard method, in which a sample is dissolved in a solvent, a small amount of reference material is added, and the solution is injected into an NMR sample tube, or the external standard method, in which a sealed capillary tube containing a solution of reference material is placed in an NMR sample tube together with a test solution. Perform measurement by installing this sample tube in the NMR probe. The test solution should be completely homogeneous. If solid foreign matter is mixed, a good-quality spectrum cannot be obtained. As a measurement solvent, a deuterated solvent for NMR measurement is usually used. Select a solvent that does not produce signals overlapping with the signal of sample, but dissolves the sample well, and does not react with the sample. In addition, caution should be taken because the chemical shift may change depending on the type of solvent, concentration of solution, concentration of deuterium ion, etc., and the resolution is reduced if the viscosity of test solution is high.

2) For the reference material, NMR measurement reagents are used. Usually, for ^{1}H and ^{13}C, tetramethylsilane (TMS) is used when organic solvents are used as measurement solvents, and sodium 3-trimethylsilylpropanesulfonate (DSS) or sodium 3-trimethylpropionate-d_4 (TSP) is used when heavy water is used.

For the other nuclei, nitromethane is used for ^{15}N, trichlorofluoromethane for ^{19}F, and phosphoric acid for ^{31}P. In addition, the residual proton in the deuterated solvent or the ^{13}C chemical shift of the measurement solvent can be used without adding a reference material.

Description of apparatus and measurement conditions

Depending on the measurement conditions, the spectrum may vary. Therefore, for appropriate comparison of the spectra, the measurement conditions such as the name of apparatus used for the measurement, the frequency of apparatus, the measurement solvent, the measurement temperature, the concentration of the test solution, the reference material, and the measurement method should be described.

Identification Method

Prepare the test solution using the method specified in the monograph and perform the test as specified in the procedure. Usually, in the case of ^{1}H NMR spectroscopy, it is identified by the following method.

A) Identification by chemical shift, multiplicity and integrated area intensity ratio

When the chemical shift, multiplicity, and integrated area intensity lof each signal of the material to be identified are specified in the monograph, the sample and the material are identified as the same when the chemical shift, multiplicity, and integrated area intensity ratio of each signal are suitable.

B) Identification by reference standard

Compare the spectrum of the sample and the reference standard under the same measurement conditions. When both

spectra have signals of the same multiplicity in the same chemical shift and have the same area intensity ratio of each signal, the sample and the reference standard are identified as the same.

Various measurement methods of ^{1}H NMR and ^{13}C NMR spectroscopy

NMR measurement methods include one- and two-dimensional, as well as three-dimensional or multidimensional NMR spectroscopy, and are used for various purposes. In one-dimensional ^{1}H NMR spectroscopy, there is spin decoupling that can eliminate coupling correlation and there is a NOE in which spatially adjacent protons are observed, and thus the space configuration or conformation can be deduced.

In one-dimensional ^{13}C NMR spectroscopy, broadband decoupling which can improve sensitivity with NOE while simplifying the spectrum, INEPT (sensitivity enhancement method for low-sensitivity nuclei by polarization shift) and DEPT (distortion-free enhancement method by polarization shift) that improve sensitivity by using the polarization shift from ^{1}H with large magnetic moment, which is directly coupled to the observation nucleus. These methods are used for determination of primary, secondary, tertiary and quaternary carbons.

In two-dimensional NMR spectroscopy, all correlation peaks between nuclei correlated by spin coupling or NOE can be observed with a single measurement, and there are many measurement methods using correlations between homonuclear species or between heteronuclear species. Typical test methods are as follows:

COSY (Correlation Spectroscopy), HOHAHA (Homonuclear Hartmann-Hahn Spectroscopy), or TOCSY (Total Correlation Spectroscopy): Chemical bond relationship of hydrogens in a molecule can be investigated by obtaining correlations between spin-coupled protons.

NOESY (NOE spectroscopy, two-dimensional NOE and chemical exchange spectroscopy): Knowledge of configuration (three dimensional structure) can be obtained by measuring the NOE effect in two dimensions and obtaining most distances between spatially close hydrogen atoms.

INADEQUATE (Incredible Natural Abundance DoublE QUAntum Transfer Experiment): Although this shows very poor sensitivity as it uses double quantum transition by spin coupling of ^{13}C - ^{13}C in natural abundance, the carbon skeleton can be directly analyzed by obtaining correlations between adjacent ^{13}C nuclei.

HMQC (Heteronuclear Multiple Quantum Coherence): This method detects the correlation between ^{1}H and ^{13}C, which are directly spin-coupled, and observes them with high sensitivity. It can show the direct chemical bond between hydrogen and carbon in a molecule.

HMBC (Heteronuclear Multiple-Bond Correlation): This method detects the correlation between ^{1}H and ^{13}C, which are remotely spin-coupled, and observes them with high sensitivity. It also shows the chemical bond relationship between hydrogen and carbon. There are several methods, including *J*-resolved two-dimensional spectroscopy, DQF-COSY (Double Quantum Filter Correlation Spectroscopy), and HSQC (Heteronuclear Single Quantum Coherence). Also, multi-dimensional NMR spectroscopy is used for macromolecules.

Optical Rotation
선광도측정법

The Optical Rotation is to measure the optical rotation of a sample with a polarimeter.

In general, the vibration of light beam occurs perpendicularly to the direction of travel, but, in ordinary light beams, the direction of vibration is not defined. However, in plane polarization, commonly called polarization, vibrations occur only in one plane including the direction of travel, so these rays are said to have a plane of polarization. Some pharmaceuticals or their solutions have a property of rotating the plane of polarized light to the right or left. This property is called optical activity or optical rotation and is related to the chemical structure of a substance.

Optical rotation is the angle at which an optically active material or its solution rotates the plane of polarized light and is measured using a polarimeter. This value is proportional to the layer length of the measuring tube and depends on the concentration, temperature and wavelength of the solution. The property of optical rotation depends on the eye of an observer when facing the light source; rotation of the polarization plane to the right is referred to as dextrorotation, and rotation to the left is referred to as levorotatory. The symbol + or - is indicated in front of the number indicating the rotation angle of the polarization plane, respectively. For example, +20° indicates a rotation to the right by 20°, and -20° indicates a rotation to the left by 20°.

Optical rotation α^{t}_{x} (°) refers to the optical rotation measured at a temperature t °C using a specific monochromatic light x (expressed as a wavelength or name), and is usually measured using the D line of the sodium spectrum as a light beam at a normal temperature of 20℃ or 25℃ and a layer length of 100 mm. However, if the wavelength specified in the monograph can be secured using an appropriate filter, other light sources such as light emitting diodes (LEDs) can be used instead.

Specific optical rotation $[\alpha]^{t}_{x}$ (°) refers to the value calculated using the optical rotation measured using the test method from the monograph. Although degrees (°) are used for convenience as a unit of specific optical rotation in the monograph, they are precisely expressed as (°·mm-1·(g/mL)−1).

The specific optical rotation $[\alpha]^{t}_{x}$ is expressed by the following formula:

$$[\alpha]^{t}_{x} = \frac{100 \cdot \alpha}{l \cdot c}$$

t: Temperature at the time of measurement

x: Wavelength or name of the specific monochromatic light in the used spectrum (Indicated as D when D line is used)

α: Angle by which the plane of polarization is rotated.

l: Layer length of the test solution, i.e., the length of the measurement tube used for measurement (mm)

c: Number of grams of drug contained in 1 mL of solution in the pharmacopeia. The density of a liquid drug when used as it is rather than as a solution. However, unless otherwise specified, the specific gravity is used instead of the density.

For example, $[\alpha]^{20}_{D}$, -33.0 to -36.0° (1 g, water, 20 mL, and 100 mm after drying) in the monograph indicates that the measurement time is -33.0 to -36.0° when measured by drying this drug under the conditions specified in the Loss on drying section, then accurately weighing about 1 g of it, adding water to dissolve it, and making 20 mL exactly.

Verification of the apparatus accuracy is verified by obtain-

ing the specific rotation value expected with the white sugar solution. A separate quartz plate is available for daily verification. If the measured value does not conform to the specifications of the quartz plate, retest after calibrating the device.

Osmolarity
삼투압측정법

The Osmolarity is to measure the osmolarity of a sample using freezing point depression.

If a pure solvent is added to a certain solution across a semipermeable membrane through which the solvent freely passes but the solute does not, a part of the solvent will osmose through the membrane into the solution. The pressure difference that occurs on both sides of the semi-permeable membrane due to the osmosis of the solvent is the osmotic pressure Π. Osmotic pressure is a physical quantity that depends on the total concentration of particles such as molecules and ions in a solution, regardless of the type of solute. Properties that depend on the total concentration of particles such as molecules, ions, etc., regardless of the type of solute, such as osmotic pressure, freezing point depression, boiling point elevation, etc. is referred to as a colligative property of a solution.

The osmotic pressure of a polymeric solution is directly measured from the change in hydrostatic pressure across a semipermeable membrane such as a cellulose membrane. However, there is no suitable semipermeable membrane that can be used to measure the osmotic pressure of a small molecular solution. Although the osmotic pressure of small molecule solutions cannot be measured directly, knowing the concentration of total particles such as molecules and ions in a solution can be used to determine the direction and magnitude of the movement of the solvent (water) through the cell membrane when the solution is under physiological conditions. The colligative property of the solution such as freezing point depression, boiling point elevation, vapor pressure depression, etc. can be easily obtained by directly measuring temperature or pressure. The colligative property of the solution, like osmotic pressure, is a quantity that depends on the total concentration of particles, and the total concentration of particle measured using this property is defined as the osmolarity. The osmolarity is defined as osmolality (mol/kg) in terms of the mass, or osmolarity (mol/L) in terms of the volume, and in practice, the osmolarity (mol/L) is normally used.

Unless otherwise specified, the as freezing point depression is used to measure osmolarity.

The freezing point depression method calculates the osmolarity (m) from the following formula between the freezing point depression ΔT (°C) and the osmolarity (m) obtained by using the property that the congealing temperature of a solution of a solute dissolved in a solvent depresses.

$$\Delta T = K \cdot m$$

In this formula, K is the molar freezing point depression constant, which is 1.86 °C kg/mol if the solvent is water.

Since the molar freezing point depression constant is defined as the mass molarity, the osmolarity(m) is obtained from the above formula, but in the dilute concentration range, this value can be considered numerically equal to the osmolarity, c (mol/L). In this test, practical osmolarity (mol/L) is used and the unit is Osm (osmol/L). 1/1000 of 1 Osm is 1 mOsm. 1 Osm indicates the concentration of particles equal to Avogadro's number (6.022×10^{23}/mol) in a 1000 mL solution. Osmolarity is usually expressed in mOsm.

Apparatus

Usually, the osmolarity is obtained by measuring the freezing point depression of water. The osmometer consists of a sample cell containing a certain amount of solution, a cooling device and a cooling bath to control the temperature, and an apparatus (thermistor thermometer) to measure the temperature electrically.

Procedure

Use a certain amount of test solution according to the used apparatus. Calibrate the osmometer (osmolar concentration) apparatus in advance using the two-point calibration method. Calibrate the apparatus by measuring the congealing temperature using 2 standard solutions for osmometer calibration that have a higher or lower osmolarity than the expected osmolarity of the sample. Additionally, if the osmolarity of the sample to be measured is less than 100 mOsm, water (0 mOsm) can be used as one of two osmolarity standard solutions. Next, rinse the sample cell and thermistor according to the method specified for the apparatus, and measure the congealing temperature with the test solution. Obtain the osmolarity (mol/kg) from the concentration dependence of the freezing point depression, and use this as the osmolarity (mol/L). In addition, if the osmolarity exceeds 1000 mOsm, dilute the sample n'/n times with water (n in n') and measure in the same way with this solution. In this case, specify that the apparent osmolarity is obtained by multiplying the value measured with n'/n-fold diluted solution by the dilution factor. Also, when diluted and measured, dilute in one step that it approaches 1000 mOsm but does not exceed 1000 mOsm. Also, if the sample is solid, dissolve it in a designated solvent and use it as the test solution.

Suitability of Apparatus

When testing the suitability of the apparatus by repeatedly measuring more than 6 times with one standard solution having a concentration close to the osmolarity of the test solution, the relative standard deviation of the test is NMT 2.0% and the deviation from the specified osmolarity is NMT 3.0%. If it is not suitable, repeat the two-point calibration and then the suitability test of the apparatus.

Preparation of osmolarity standard solution for apparatus calibration

Dry sodium chloride (standard reagent) at 500 ~ 650 °C for 40 ~ 50 minutes, and then cool in a desiccator (silica gel). Accurately weigh the amount of sodium chloride corresponding to each osmolarity standard solution in the following table and dissolve it in 100 g of water to obtain each osmolarity standard solution.

Osmolarity standard solution for apparatus calibration	Amount of sodium chloride (g)
100 mOsm standard solution	0.309
200 mOsm standard solution	0.626
300 mOsm standard solution	0.946
400 mOsm standard solution	1.270
500 mOsm standard solution	1.593
700 mOsm standard solution	2.238
1000 mOsm standard solution	3.223

Osmolarity ratio

In this measurement, the ratio of the osmolarity of the test solution to the osmolarity of the normal saline injection solution is defined as the osmolarity ratio, and is used as a measure of isotonicity. Since the osmolarity c_S (mOsm) of a normal saline

injection solution (0.900 g/100 mL) is constant (286 mOsm), measure the osmolarity c_T (mOsm) of the test solution and calculate its osmolarity ratio using the following formula:

$$\text{Osmotic pressure ratio} = \frac{C_T}{C_S}$$

C_S: 286 mOsm

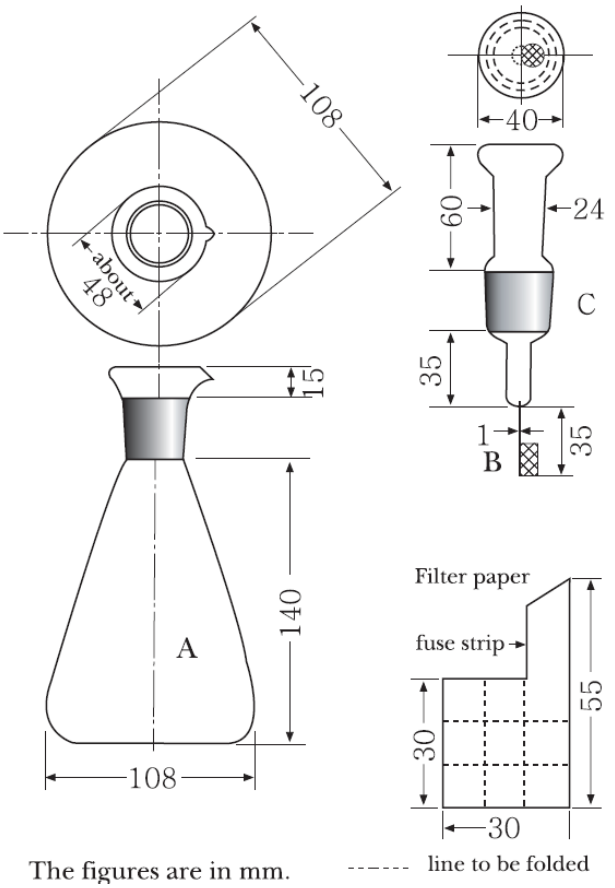
In addition, if a sample exceeding 1000 mOsm is diluted and measured, the dilution factor is n'/n and the measured osmolarity is c'_T, calculate the osmotic pressure ratio using the formula $n'/n \cdot c'_T = c_T$, assuming the linearity of osmolarity against solute concentration. However, when diluted and measured, the dilution ratio is specified as (n in n').

Oxygen Flask Combustion
산소플라스크연소법

The Oxygen Flask Combustion entails the combustion of organic compounds containing chlorine, bromine, iodine, fluorine, sulfur, and other elements within a flask filled with oxygen. This process allows to identify and quantify halogen, sulfur, and other elements present in the compound.

Apparatus

Use the apparatus shown in the following figure.



A: Colorless, thick-walled (about 2 mm), 500 mL hard glass flask, opened slightly upward. However, a flask made of quartz should be used for the quantification of fluorine.

B: Platinum basket or cylinder made of welded platinum gauze (It is hung at the lower end of the stopper C with platinum wire)

C: Ground stopper made of hard glass. However, for the quantification of fluorine, a flask made of quartz should be used.

Preparation of test solution and blank test solution

Unless otherwise specified, proceed by the following method.

1) Sample preparation

A) For solid samples

Accurately weigh the amount of the sample specified in the monograph, place it on the center of the filter paper as shown in the figure, wrap the paper carefully along the dotted line to prevent it from scattering, and place the parcel in a platinum basket or cylinder B, leaving its fuse-strip on the outside.

B) For liquid samples

Roll up a suitable amount of absorbent cotton in advance with filter paper 50 mm length and 5 mm width, leaving the end part of the paper for a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder B. Collect the sample using an appropriate glass column, weigh its mass accurately, and put one end in contact with an absorbent cotton to infiltrate the amount of sample specified in the monograph.

2) Combustion method

Put the absorbent liquid specified in the monograph into flask A and fill A with oxygen in advance. Then, wet the ground part of stopper C with water, ignite the ignition part, and immediately insert it into A and keep it sealed until combustion is complete. Then, shake the flask occasionally until the white smoke in A completely disappears, then allow to stand for 15 to 30 minutes, and use it as the test solution. Separately, prepare a blank test solution by proceeding in the same way without using a separate sample.

Procedure of determination

Unless otherwise specified in the monograph, proceed by the following method.

1) Chlorine or bromine

Add a small amount of water to the top of A, carefully open the stopper C, and transfer the test solution to the beaker. Wash the inner walls of C, B, and A with 15 mL of 2-propanol and add the washed solution to the test solution. Add 1 drop of bromophenol blue TS to this solution and add diluted nitric acid dropwise until the solution turns yellow. Then, add 25 mL of 2-propanol and titrate with 0.005 mol/L silver nitrate VS using the potentiometric titration of the endpoint detection. Perform the test with the blank test solution in the same way, and make any necessary correction.

1 mL of 0.005 mol/L silver nitrate VS = 0.17727 mg of Cl
1 mL of 0.005 mol/L silver nitrate VS = 0.39952 mg of Br

2) Iodine

Add a small amount of water to the top of A, carefully open stopper C, and add 2 drops of hydrazine monohydrate to the test solution. Close stopper C and shake vigorously and mix to decolorize. Transfer the contents of A to a beaker, wash C, B, and the inner walls of A with 25 mL of 2-propanol, and transfer the washed solution to the beaker. Add 1 drop of bromophenol blue TS to this solution and add diluted nitric acid dropwise until the

solution turns yellow. Then, titrate with 0.005 mol/L silver nitrate VS using the potentiometric titration of the endpoint detection. Perform the test with the blank test solution in the same way, and make any necessary correction.

1 mL of 0.005 mol/L silver nitrate VS = 0.6345 mg of I

3) Fluorine

Add a small amount of water to the top of A and carefully open the stopper C. Transfer the test solution and blank test solutions to a 50 mL volumetric flask respectively, wash the inner walls of C, B, and A with water, and add the washed solution to the test solution with water to make 50 mL. Use the resulting solution as the test solution and calibration solution. Take exactly V mL of the test solution, V mL of the calibration solution, and 5 mL of the fluorine standard solution, all equivalent to about 30 μg of fluorine, into separate 50 mL volumetric flasks and shake well. To each of them, add 30 mL of an alizarin complexone mixture, pH 4.3 acetic acid/potassium acetate buffer, and cerium (III) nitrate TS (1:1:1). Add water to make 50 mL, and allow to stand for 1 hour. Manipulate these solutions in the same manner with 5 mL of water, and use the resulting solution as the blank and test it according to the Ultraviolet-visible Spectrophotometry. Measure the absorbance A_T, A_C, and A_S of the test solution, the calibration solution, and the standard solution respectively at a wavelength of 600 nm.

Amount (mg) of fluorine (F) in the test solution
= Amount (mg) of fluorine in 5 mL of the standard solution
$\times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$

Fluorine standard solution: Put sodium fluoride (standard reagent) into a platinum crucible and dry it at 500 to 550 ℃ for 1 hour. Then, allow to cool in a desiccator (silica gel), accurately weigh about 66.3 mg, and dissolve in water to make exactly 500 mL. Take exactly 10 mL of this solution and add water to make exactly 100 mL.

4) Sulfur

Add a small amount of water to the top of A, carefully open the stopper C, and wash the inner walls of C, B, and A with 15 mL of methanol. To this solution, add 40 mL of methanol, then add exactly 25 mL of 0.005 mol/L barium perchlorate VS, and let stand for 10 minutes. Add 0.15 mL of Arsenazo III TS with a graduated pipette, and titrate with 0.005 mol/L sulfuric acid VS. Perform the test with the blank test solution in the same way, and make any necessary correction.

1 mL of 0.005 mol/L barium perchlorate VS = 0.16033 mg of S

Paper Chromatography
여지크로마토그래프법

The Paper Chromatography is a technique in which a mixture is developed into a mobile phase (solvent) using a filter paper (stationary phase) to separate its individual components based on their different distribution between the two phases, and is used to identify substances or to test purity.

Procedure Unless otherwise specified, proceed by the following method.

Dissolve the substances to be analyzed in a suitable solvent to prepare the test solution. Mark a starting line at a height of about 5 cm from the bottom end of a rectangular filter paper measuring 2 to 3 cm and about 40 cm in length. At the center of the starting line, drop the amount of test solution specified in the monograph using a micropipette or capillary tube, and allow it to air-dry. Next, in a developing chamber of about 50 cm in height that has been previously filled with a developing solvent and saturated with vapor, hang the filter paper carefully so that it does not touch the chamber wall. Dip the paper into the mobile phase until about 1 cm from its, seal the chamber and allow the filter paper to develop at ordinary temperature.

When the solvent tip has reached the distance specified in the monograph from the starting line, take the filter paper out of the chamber, quickly mark the solvent tip, and air-dry the paper. Then, compare the R_f value, color, etc., of the spots according to the method specified in the monograph. Calculate the R_f value by using the following equation:

$$R_f = \frac{\text{Distance from the starting line to the center of the spot}}{\text{Distance from the starting line to the solvent tip}}$$

The different substances are identified according to their Rf value. The R_f value is specific and constant for a particular substance and reflects its distribution coefficient under standard conditions.

Paramagnetic Oxygen Analysis
산소분석법

Oxygen in gases is determined using a paramagnetic analyzer. The principle of the method is based on the paramagnetic characteristics of the oxygen molecule. The paramagnetic analyzer converts the reaction of oxygen in a magnetic field into an electrical signal and measures the oxygen concentration based on the principle that the electrical signal and the oxygen concentration are proportional to each other. This method is sensitive to pressure and temperature, so it must be corrected with standard gas immediately before use, and the measurement sensitivity is 0.1% or less.

Calibration

Set to zero by passing a certified standard nitrogen gas through the analyzer at a fixed flow rate until a constant reading is obtained. Separately, set the scale to 100% by passing a certified standard oxygen through the analyzer at the same flow rate as the nitrogen until a constant reading is obtained.

Assay

Pass the sample gas through the analyzer at a fixed flow rate and measure the obtained constant values obtained.

Note

Use the following certified standard gases in this analysis.

1) Standard oxygen (paramagnetic gas), O_2

High purity grade oxygen (content NLT 99.99 vol%) CRM 112-06-002, prescribed by KRISS (Korea Research Institute of Standards and Science)

2) Standard nitrogen (diamagnetic gas), N_2

High purity grade nitrogen (content NLT 99.99 vol%) CRM 112-06-003, prescribed by KRISS (Korea Research Institute of Standards and Science)

Particle Size Distribution Estimation by Analytical Sieving
제제의 입도시험법

The Particle Size Distribution Estimation by Analytical Sieving is a method to determine the particle size of granules and powders.

Procedure

Unless otherwise specified, the test is performed according to the following procedures. If necessary, it can be determined separately.

1) Granules

Granules are tested using No. 10 (1700 μm), No. 12 (1400 μm), and No. 42 (355 μm) sieves. The internal diameter of the sieves used in this test method is 75 mm. Accurately weigh20.0 g of granules and place it on the top (coarsest) sieve of a container, which includes the sieves specified above, followed by a receptacle. Cover it with a lid, shake horizontally for 3 minutes, occasionally tapping gently, and then weigh the residue mass in each sieve and receptacle. This preparation is considered to meet the requirements when all of the granules pass through the No. 10 (1700 μm) sieve, ≤ 5% of the entire granules remain on the No. 12 (1400 μm) sieve, and ≤ 15% of the entire granules pass through the No. 42 (355 μm) sieve.

2) Powders

Powders are -tested using No. 18 (850 mm), No. 30 (500 mm), and No. 200 (75 mm) sieves. The internal diameter of the sieves used in this test method is 75 mm. Accurately weigh10.0 g of powders and place it on the top (coarsest) sieve of a container, which includes the sieves specified above, followed by a receptacle. Cover it with a lid, shake horizontally for 3 minutes, occasionally tapping gently, and then weigh the residue mass in each sieve and receptacle. This preparation is considered to meet the requirements when all of the powder passes through the No. 18 (850 μm) sieve and ≤ 5% of the entire powder remains on the No. 30 (500 μm) sieve. However, it is considered to meet the requirements for fine particles ≤ 10% of the entire powder passes through the No. 200 (75 μm) sieve.

Particulate Contamination: Visible Particles
불용성이물시험법

The Particulate Contamination: Visible Particles is a method to examine the presence of foreign insoluble matters in ophthalmic solutions and injections.

Ophthalmic solutions

Aqueous ophthalmic solution and aqueous solvent for ophthalmic solutions dissolved before use, should appear clear and free from readily detectable foreign insoluble matter when observed with the naked eye at a luminous intensity of 3000 to 5000 lux using a white light source.

Injections

Method 1

Injectable solution and solvent for injections dissolved before use should appear clear and free from readily detectable foreign insoluble matter when observed with the naked eye at a light intensity of approximately 1000 lux using a white light source after cleaning the exterior of the container. For plastic containers, the test should be performed by observing with the naked eye at a light intensity of approximately 8000 to 10,000 lux using a white light source above and below the container.

Method 2 For injections dissolved before use, clean the exterior of containers and carefully dissolve the content with the specified solvent or water for injection, while avoiding any contamination with foreign insoluble matters. The solution must be clear and free from readily detectable foreign insoluble matter when visually inspected at a light intensity of approximately 1000 lux using a white light source.

Peptide Mapping
단백질의 펩티드 지도작성법

The Peptide Mapping is a method used for the Identification of proteins, especially those manufactured using recombinant DNA (r-DNA) technology. It involves the chemical or enzymatic cleavage of proteins, to generate peptide fragments, which are then separated and identified using a reproducible analytical approach. It is a particularly valuable for detecting single amino acid changes that may occur due to factors such as errors in the reading complementary DNA (cDNA) sequences or point mutations. Peptide mapping is a comparative method, where the information obtained is compared to a reference standard or similarly treated reference material. This comparison confirms the primary structure of the protein detects structural alterations, and provides evidence of process consistency and genetic stability. Each protein presents unique characteristics which must be well understood so that the scientific and analytical approaches permit validated development of a peptide map that provides sufficient specificity.

This chapter provides detailed assistance in the application of peptide mapping and its validation to characterize the desired protein product, to evaluate the stability of the expression construct of cells used for recombinant DNA products, to evaluate the consistency of the overall process and to assess product stability as well as to ensure the identity of the protein product, or to detect the presence of protein variant.

1. Peptide Map

Peptide mapping is not a generic method, instead, it entails the development of specific maps for each unique protein. While the technology is evolving rapidly, several well-established methods are generally accepted. Variations of these methods will be indicated, when appropriate, in specific monographs. A peptide map may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provide a comprehensive understanding of the protein being analysed. Four major steps are necessary for the development of the procedure: isolation and purification of the protein, if it is part of a formulation; selective cleavage of the peptide bonds; chromatographic separation of the peptides; and analysis and identification of the peptides. A sample is digested and assayed in parallel with a reference standard or a reference material. Complete cleavage of peptide bonds is more likely to occur when enzymes, such as endoproteases (e.g., trypsin), are used, instead of chemical cleavage reagents. A map should contain enough peptides to be meaningful. On the other hand, if there are too many fragments, the map might lose its specificity because many proteins will then have

the same profiles.

2. Isolation and Purification

Isolation and purification are necessary for analysis of bulk drugs or dosage forms containing interfering excipients and carrier proteins. If necessary, these processes will be specified in the monograph. When required, the quantitative recovery of proteins from the dosage form should be validated.

3. Selective Cleavage of Peptide Bonds

The selection of the approach used for cleavage of peptide bonds will depend on the specific protein under test. This selection process involves determining the type of cleavage to be employed, either enzymatic or chemical, and the choice of cleavage agent within the selected category. Several cleavage agents and their specificities are listed in Table 1. This list is not exhaustive and may be expanded as new cleavage agents are identified.

Table 1. Examples of cleavage agents

Type	Agent	Specificity
Enzymatic	Trypsin, EC 3.4.21.4	*C*-terminal side of Arg and Lys
	Chymotrypsin, EC 3.4.21.1	*C*-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin, EC 3.4.23.1 and EC 3.4.23.2	Nonspecific digest
	Lysyl endopeptidase (Lys-C endopeptidase), EC 3.4.21.50	*C*-terminal side of Lys
	Glutamyl endopeptidase; (from *S. aureus* strain V8), EC 3.4.21.19	*C*-terminal side of L-Glu and Asp
	Peptidyl-Asp metalloendopeptidase (Asp-N endoproteinase), EC 3.4.24.33	*N*-terminal side of Asp
	Clostripain, EC 3.4.22.8	*C*-terminal side of Arg
Chemical	Cyanogen bromide	*C*-terminal side of Met
	2-Nitro-5-thiocyanobenzoic acid	*N*-terminal side of Cys
	o-Iodosobenzoic acid	*C*-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	BNPS-skatole	Trp

3.1. Pretreatment of Sample

Depending on the size or configuration of the protein, various sample pretreatment methods can be employed. In the case of monoclonal antibodies, the heavy and light chains must be separated before cleavage for peptide mapping. If trypsin is used as a cleavage agent for proteins with a molecular weight greater than 100,000 Da, it is essential to protect lysine residues through citraconylation or maleylation to prevent the generation of excessive peptide fragments.

3.2. Pretreatment of the Cleavage Agent

Pretreatment of cleavage agents, particularly enzymatic agents, may be required for purification purposes to ensure reproducibility of the peptide map. For example, trypsin used as a cleavage agent will have to be treated with tosyl-L-phenylalanine chloromethyl ketone(TPCK) to inactivate chymotrypsin.

Other methods, such as HPLC-based trypsin purification or immobilizing enzymes on a gel support, have been successfully applied when dealing with a limited amount of protein.

3.3. Pretreatment of the Protein

Under certain conditions, such as when sample concentration is low, it might be necessary to concentrate the sample or to separate the protein from added substances and stabilizers used in formulation of the product, if these interfere with the mapping procedure. Physical pretreatment methods include ultrafiltration, column chromatography, and lyophilization. Other pretreatments, such as the addition of chaotropic agents (e.g., urea) can be used to unfold the protein prior to mapping. To allow the enzyme to have full access to cleavage sites and permit some unfolding of the protein, it is often necessary to reduce and alkylate the disulfide bonds prior to digestion.

When digesting with trypsin, it is important to be aware of potential side reactions that can impact the clarity of the map, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, or the formation of pyroglutamic groups resulting from the deamidation of glutamine at the N-terminal side of a peptide. Furthermore, peaks may be produced by autohydrolysis of trypsin. Their intensities depend on the ratio of trypsin to protein. To avoid autohydrolysis, solutions of proteases may be prepared at a pH that is not optimal (e.g., at pH 5 for trypsin), which would mean that the enzyme would not become active until diluted with the digest buffer.

3.4. Establishment of Optimal Digestion Conditions

Factors that affect the completeness and effectiveness of protein digestions can influence various chemical or enzymatic reactions.

(i) pH: The pH of the digestion mixture is empirically determined to ensure the optimal performance of the given cleavage agent. For example, when using cyanogen bromide as a cleavage agent, a highly acidic environment (e.g., pH 2 with formic acid) is necessary. However, when using trypsin as a cleavage agent, a slightly alkaline environment (pH 8) is optimal. As a general rule, the pH of the reaction medium remains constant during the digestion, maintaining the chemical integrity of the protein and ensuring it doesn't change throughout the fragmentation reaction.

(ii) Temperature: A temperature between 25°C and 37°C is adequate for most digestions. The temperature used is intended to minimize chemical side reactions. The type of protein under test will dictate the temperature of the reaction medium because some proteins are more susceptible to denaturation as the temperature of the reaction increases.

For example, digestion of recombinant bovine somatropin is conducted at 4°C because it precipitates at higher temperatures during digestion.

(iii) Time: If sufficient sample is available, consider a time course to determine the optimum time for obtaining a reproducible map and avoiding incomplete digestion. The digestion time can vary from 2 to 30 hours. The reaction can be stopped by the adding an acid that does not interfere with the tryptic map or by freezing the reaction.

(iv) Amount of cleavage agent: Although excessive amounts of cleavage agent are used to achieve reasonably rapid digestion time (i.e., 6 to 20 hours), the amount of cleavage agent should be minimized to avoid its impact on the chromatographic map pattern. Typically, a protein-to-protease ratio between 20:1 and 200:1 is used. It is recommended to add cleavage in two or more stages to optimize cleavage. Nonetheless, the final reaction volume should remain small to facilitate the next step in peptide mapping —the separation step. To eliminate digestion artifacts that might interfere with subsequent analysis, perform a blank determination using a digestion control with all the reagents, except the test protein.

4. Chromatographic Separation

Many techniques are used to separate peptides for mapping. The selection of a technique depends on the protein being mapped. Techniques that have been successfully used for peptide separation are listed in Table 2. In this section, one of the most widely used methods for chromatographic separation, reverse-phase high performance liquid chromatography (RP-HPLC), is described as one of the procedures of chromatographic separation.

Table 2. Techniques used for the separation of peptides

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)
Ion-Exchange Chromatography (IEC)
Hydrophobic Interaction Chromatography (HIC)
Polyacrylamide Gel Electrophoresis (PAGE), nondenaturating
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
Capillary Electrophoresis (CE)
Paper Chromatography—High Voltage (PCHV)
High-Voltage Paper Electrophoresis (HVPE)

The purity of solvents and mobile phases is a critical factor in HPLC separation. Commercially available HPLC-grade solvents and water are recommended for reversed-phase high-performance liquid chromatography (RPHPLC). Dissolved gases can pose issues in gradient systems where the solubility of the gas in a solvent may be lower in a mixture than in a single solvent. Vacuum degassing and sonication are commonly employed degassing methods. When solid particles from the solvents are introduced into the LC system, they can damage the sealing of pump valve seals or obstruct the front end of the chromatographic column. Therefore, it is advisable to use filtration before and after the pump.

4.1. Chromatographic column

The selection of a chromatographic column depends on the characteristics of the protein being separated. For example, columns with pore sizes of 100 Å or 300 Å and made of silica gel provide the best separation. For smaller peptides, octylsilane chemically bonded to totally porous silica articles, 3 to 10 mm in diameter (C8) and octadecylsilane chemically bonded to porous silica 3 to 10 µm in diameter (C18) column packings are more efficient than the butyl silane chemically bonded to totally porous silica particles, 5 to 10 µm in diameter (C4) packing.

4.2. Solvent

The most commonly used organic solvent is acetonitrile mixed with water, with less than 0.1% trifluoroacetic acid added. If necessary, isopropanol or n-propanol can be added to enhance solubility, provided that it does not significantly increase the solution's viscosity.

4.3. Mobile phase

Buffered mobile phases containing phosphate are used to provide some flexibility in the selection of pH conditions, since shifts of pH in the 3.0 to 5.0 range enhance the separation of peptides containing acidic residues (e.g., glutamic and aspartic acids). Sodium or potassium phosphates, ammonium acetate, phosphoric acid, and a pH between 2 and 7 (or higher for polymer-based packing materials) have also been used with acetonitrile gradients. Acetonitrile containing trifluoroacetic acid is used quite often.

4.4. Gradient selection

Gradients can be linear, nonlinear, or include step functions. A shallow gradient is recommended for separating complex mixtures. Gradients should be optimized to ensure clear resolution of one or two peaks that will serve as “marker” peaks for the test.

4.5. Isocratic selection

Isocratic HPLC systems using a single mobile phase are favoured for their ease of use and improved detection sensitivity. However, establishing the optimal composition of the mobile phase to achieve clear resolution of each peak can sometimes be challenging. Mobile phases that are sensitive to slight changes in component ratios or pH, resulting in significant variations in peptide retention times, should not be used in isocratic HPLC systems.

4.6. Other parameters

Temperature control of the column is essential to achieving good reproducibility. Mobile phase flow rates typically range from 0.1 to 2.0 mL per minute, and peptide detection is carried out using a UV detector set at 200 to 230 nm. While alternative detection methods, such as postcolumn derivatization, have been employed, they do not match the robustness and versatility of UV detection.

4.7 Validation

This section outlines an experimental approach for assessing the overall performance of the test method. The acceptance criteria for system suitability depend on the identifying of critical test parameters that affect data interpretation and acceptance. These critical parameters are also served as criteria for monitoring peptide digestion and peptide analysis. To confirm whether the desired digestion endpoint has been reached, a comparison with a reference standard or reference material, treated identically to the sample under test, is performed. It is crucial to use a reference standard or reference material in parallel with the test protein when developing and establishing system suitability limits. Additionally, a sample chromatogram should be provided for comparison with the reference standard or reference. Other indicators may include visual inspection of protein or peptide solubility, the absence of intact protein, or measurement of responses to digestion-dependent peptides.

The specific system suitability parameters for peptide analysis depend on the chosen mode of peptide separation and detection, as well as the data analysis requirements.

When peptide mapping serves as the Identification, the system suitability requirements for the identified peptides covers selectivity and precision. In such cases, as well as when identifying variant proteins, verifying the primary structure of the peptide fragments in the peptide map confirms both the known primary structure and the identifies protein variants by comparing with the peptide map of the reference standard or reference material for the specified protein. The use of a digested reference standard or reference material for a given protein is the preferred method for assessing peptide. In the analysis of a variant protein, a characterized mixture of a variant and a reference standard or reference material can be used, particularly if the variant peptide is located in a less-resolved region of the map. The index of pattern consistency can be determined by assessing peak-to-peak resolution, maximum peak width, peak area, peak tailing factors, and column efficiency among other chromatographic parameters.

Depending on the protein under test and the chosen separation method, single peptide or multiple peptide resolution requirements may be necessary.

Replicate analysis of the digest of the reference standard or reference material for the protein under test provides measures of precision and quantitative recovery. Recovery of the identified peptides is typically evaluated using internal or external peptide standards, with precision expressed as the relative standard deviation (RSD). As differences in the recovery and precision of the identified peptides are expected, system suitability limits should be established for both the recovery and precision, unique to each protein, as specified in the individual monograph.

The initial examination involves visual comparisons of relative retention times, peak responses (peak area or peak height), the number of peaks, and the overall elution pattern.

This is followed by mathematical analysis of the peak response ratios and the chromatographic profile of a 1:1 (v/v) mixture of sample and reference standard or reference material digest. If all peaks in the sample and reference standard or reference material digest have the same relative retention times and peaks response ratios, then the identity of the sample under test is confirmed.

If peaks that initially eluted at significantly different relative retention times appear as single peaks in the 1:1 mixture, the initial difference indicates system variability. However, if separate peaks are observed in the 1:1 mixture, this would be evidence of the nonequivalence of the peptides in each peak. If a peak in the 1:1 mixture is significantly broader than the corresponding peak in the sample and reference standard or reference material digest, it may indicate the presence of different peptides.

The use of computer-aided pattern recognition software for the analysis of peptide mapping data has been proposed and applied. Nevertheless, issues related to the validation of the computer software prevent its immediate use in a compendial test. Other automated approaches that employ mathematical formulas, models, and pattern recognition have been used, such as the automated identification of compounds by IR spectroscopy and the application of diode-array of Ultraviolet-visible Spectroscopy for peptides identification. These methods have limitations due to inadequate resolution, co-elution of fragments, or absolute peak response differences between reference standard or reference material and sample fragments.

The numerical comparison of the retention times and peak areas or peak heights can be performed for a selected group of relevant peaks that have been correctly identified in the peptide maps. Peak areas can be calculated using one peak with relatively small variation as an internal reference, keeping in mind that peak area integration is sensitive to baseline variation and can introduce error into the analysis.

Alternatively, the percentage of each peptide peak height relative to the sum of all peak heights can be calculated for the sample under test and compared to that of the corresponding peak in the reference standard or reference material. Auto-hydrolysis of trypsin is monitored by producing a blank peptide map, which is the peptide map obtained when a blank test solution is treated with trypsin.

The minimum requirement for the qualification of peptide mapping is an approved test procedure that includes system suitability as a test control. In the early stages of regulation, qualification of peptide mapping for a protein is typically sufficient. As the regulatory approval process for the protein advances, additional qualifications of the test may be needed, including partial validation of the analytical procedure to ensure that the method will perform as intended in the development of a peptide map for the specified protein.

5. Analysis and Identification of Peptides

This section gives guidance on the use of peptide mapping during development in support of regulatory applications. The use of a peptide map as a qualitative tool does not require the complete characterization of the individual peptide peaks. However, validation of peptide mapping in support of regulatory applications requires rigorous characterization of each of the individual peaks in the peptide map. Methods to characterize peaks range from N-terminal sequencing of each peak followed by amino acid analysis to the use of mass spectroscopy (MS).

For characterization purposes, when employing N-terminal sequencing and amino acids analysis, the analytical separation is expanded. Since scaling-up may impact the resolution of peptide peaks, it is imperative, based on empirical data, demonstrate that no loss of resolution due to scale-up. Eluates corresponding to specific peptide peaks are collected, vacuum-concentrated, chromatographed, and, if necessary, subjected to chromatography once more. Amino acid analysis of fragments may be constrained by the size of the peptide. If the N-terminus is blocked, clearance before sequencing may be required. Additionally, C-terminal sequencing of proteins in combination with carboxypeptidase digestion and MALDI-TOF MS can also be employed for characterization purposes.

Characterization of peptide fragments via MS can be performed either by directly infusing isolated peptides or by conducting structure analysis through online LC-MS. This generally includes electrospray and MALDI-TOF MS analysers as well as fast atom bombardment (FAB). Tandem MS has also used to determine the sequence a modified proteins and identify the type of amino acid modifications that have occurred. Comparing mass spectra of digests before and after reduction allows for the localization of disulfide bonds in various sulfhydryl-containing peptides.

If regions of the primary structure are not clearly demonstrated by the peptide map, it might be necessary to develop a secondary peptide map. The goal of a validated method of characterization of a protein through peptide mapping is to reconcile and account for at least 95% of the theoretical composition of the protein structure.

pH Measurement
pH 측정법

pH is defined as the common logarithm of the reciprocal of hydrogen ion activity, which is obtained by multiplying the hydrogen ion concentration in an aqueous solution by the activity coefficient. Practically, it is used as a scale to measure the hydrogen ion concentration in a test solution.

The pH of a test solution is expressed as the following equation, regarding to the pH of a standard solution (pHs) and can be measured using a pH meter with a glass electrode.

$$\mathrm{pH} = \mathrm{pH_S} + \frac{\mathrm{E} - \mathrm{E_S}}{2.3026\ \mathrm{RT/F}}$$

$\mathrm{pH_S}$: pH of a standard pH solution

E: Electromotive force (V) of the cell with a combination of glass and reference electrodes in a test solution; the constitution of the cell is as follows:

Glass electrode | test solution | reference electrode

ES: Electromotive force (V) of the cell with a combination of glass and reference electrodes in a standard pH solution; the

constitution of the cell is as follows:

Glass electrode | standard pH solution | reference electrode

R: Gas constant
T: Thermodynamic temperature
F: Faraday constant

In the above equation, 2.3026 RT/F represents the electromotive force (V) per pH unit, and depends on temperature as shown in the Table 1.

Standard pH solution

Standard pH solutions are used for a reference of pH. To prepare it, boil distilled water or water with a conductivity of less than 2 $\mu S \cdot cm^{-1}$ (25 °C) and organic carbon of less than 0.50 mg/L for at least 15 minutes, and evaporate carbon dioxide. Cool in a container fitted with a carbon dioxide-absorbing tube (soda lime). Prepare standard pH solutions as specified in Table 2. Store the standard pH solutions in airtight hard glass or polyethylene bottles. Basic standard solutions are recommended to store in containers fitted with a carbon dioxide-absorbing tube. As the pH may change over prolonged storage, compare the pH of old solutions with new ones to make sure that they are same before use.

Table 1. Temperature dependence of electromotive force

Temperature of solution (°C)	2.3026 RT/F (V)
5	0.05519
10	0.05618
15	0.05717
20	0.05817
25	0.05916
30	0.06015
35	0.06114
40	0.06213
45	0.06313
50	0.06412
55	0.06511
60	0.06610

Oxalate Standard pH Solution

Powder the hydrogen dioxalate dihydrate for pH Measurement and dry it in a desiccator (silica gel). Weigh exactly 12.71 g (0.05 mole) and dissolve in water to obrain an exact total volume of 1000 mL.

Phthalate Standard pH Solution

Powder the potassium hydrogen phthalate for pH Measurement and dry it at 110 °C until a constant mass is achieved. Weigh exactly 10.21 g (0.05 mole) and dissolve in water to obtain an exact total volume of 1000 mL.

Phosphate Standard pH Solution

Powder the potassium dihydrogen phosphate for pH measurement and disodium hydrogen phosphate for pH measurement. Dry them at 110 °C until a constant mass is achieved. Weigh exactly 3.40 g (0.025 mole) of potassium dihydrogen phosphate and 3.55 g (0.025 mole) of disodium hydrogen phosphate and dissolve in water to obtain an exact volume of 1000 mL.

Borate Standard pH Solution

Place the sodium borate for pH Measurement in a desiccator (sodium bromide saturated solution) until a constant mass is achieved. Weigh exactly 3.81 g (0.01 mole) and dissolve in water to obtain an exact total volume of 1000 mL.

Carbonate Standard pH Solution

Dry sodium hydrogen carbonate for pH Measurement in a desiccator (silica gel) until a constant mass is achieved and weigh exactly 2.10 g (0.025 mole) of it. Dry sodium carbonate for pH Measurement between 300 °C and 500 °C until a constant mass and weigh exactly 2.65 g (0.025 mole) of it. Dissolve both reagents in water to obtain an exact total volume of 1000 mL.

Calcium Hydroxide Standard pH Solution

Powder the calcium hydroxide for pH Measurement and transfer 5 g to a flask. Add 1000 mL of water and shake well. Ensure the temperature remains between 23 °C and 27 °C. Saturate the mixture thoroughly with water, and filter the supernatant at the same temperature. Utilize the clear filtrate, which should be about 0.02 mol/L.

The pH values of these pH solutions at various temperatures are provided in Table 2. pH values at temperatures not listed can be calculated through interpolation.

Table 2. Temperature dependence of pH for standard solutions

Temperature (°C)	Oxalate	Phthalate	Phosphate	Borate	Carbonate	Calcium hydroxide
	standard pH solution					
0	1.67	4.01	6.98	9.46	10.32	13.43
5	1.67	4.01	6.95	9.39	10.25	13.21
10	1.67	4.00	6.92	9.33	10.18	13.00
15	1.67	4.00	6.90	9.27	10.12	12.81
20	1.68	4.00	6.88	9.22	10.07	12.63
25	1.68	4.01	6.86	9.18	10.02	12.45
30	1.69	4.01	6.85	9.14	9.97	12.30
35	1.69	4.02	6.84	9.10	9.93	12.14
40	1.70	4.03	6.84	9.07		11.99
50	1.71	4.06	6.83	9.01		11.70
60	1.73	4.10	6.84	8.96		11.45

Apparatus

A typical pH meter consists of a detection unit with both of glass and reference electrodes, an amplifier that amplifies the detected electromotive force, and a display unit to showcase the measured results. This display unit is equipped with dials for zero point correction and sensitivity adjustment. Some models may also feature a temperature compensation dial. The reproducibility of pH meter should deviate NMT 0.05 pH unit when measuring the pH value of any of the standard solutions listed above five times consecutively, after thoroughly ringing the detection unit with water between measurements.

Procedure

Immerse the glass electrode in water for more than several hours in advance. Make sure that the pH meter is stable after turning on the power before use. Rinse the detection unit with water and dab away any residual water with filter paper.

To calibrate the pH meter, two types of standard pH solutions are typically used. Immerse the electrode in the phosphate standard pH solution and adjust the display to match the expected pH shown in the table using the zero point correction dial. Then, measure the pH under identical conditions using the standard pH solution with around expected pH of the test solution as the second standard solution. If the observed pH does not match the pH shown in the table, make the necessary adjustments using the sensitivity adjustment dial. Repeat this process until both standard solutions consistently display the pH values within the specified range, with a tolerance of ± 0.05 pH unit, without any adjustment. If the pH meter comes with a temperature compensation dial,

align the temperature value with that of the standard solution before calibration. When using an apparatus which can perform the above calibration automatically, make sure that periodically verify that the pH readings of two standard pH solutions fall within the specified range, with a tolerance of ± 0.05 pH unit. After calibrating, thoroughly rinse the detection unit with water and blot away any remaining moisture using filter paper.

Immerse the detection unit in the test solution. Once the display stabilizes, record the pH. If necessary, the test solution can be gently stirred. In addition, ensure that the test solution's temperature is same (within ± 2 ℃) with the standard solutions used for calibration. For alkaline test solutions, use a container with a lid so that measurements can be done under inert gas environment, such as nitrogen. Alkaline solutions with metallic ions at pH 11 or higher might produce greater discrepancies. In these instances, use an electrode with a lower alkali error, and recalibrate as necessary.

Plastic Containers for Pharmaceutical Use
플라스틱제의약품용기시험법

The Plastic Containers for Pharmaceutical Use can be utilized to design and assure the quality of plastic containers for pharmaceutical products. It is not obligatory to conduct all of the following tests on every pharmaceutical containers. As this test method might not encompass all the necessary test for the design and evaluation of the quality of plastic containers for pharmaceutical products, additional tests should be incorporated if necessary.

Combustion tests

1) Residue on ignition

Accurately weigh 5 g of cut pieces of the container and perform the test according to the Residue on Ignition.

2) Heavy metals

Place an appropriate amount of cut pieces of the container in a porcelain crucible, and perform the test according to Method 2 of the Heavy Metals. Prepare the control solution with 2.0 mL of lead standard solution.

3) Lead

Method 1

Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, soak them with 2 mL of sulfuric acid, heat slowly to dryness, and heat to combustion at 450 °C – 500 °C. Repeat the above procedure if necessary. After cooling it down, moisten the residue with water, add 2 – 4 mL of hydrochloric acid to evaporate the residue to dryness on a water bath, then add 1 – 5 mL of hydrochloric acid, and dissolve it by heating. Then add 0.5 – 1 mL of a mixture of citric acid monohydrate (1 in 2) solution and hydrochloric acid (1 : 1), and add 0.5 – 1 mL of heated ammonium acetate solution (2 in 5). If there is any insoluble matter remaining, filter through a glass filter (G3). To this filtrate, add 10 mL of diammonium hydrogen citrate solution (1 in 4), 2 drops of bromothymol blue TS, and ammonia TS until the color of the solution changes from yellow to green. Then, add 10 mL of a ammonium sulfate solution (2 in 5) and water to make 100 mL. Add 20 mL of sodium *N*,*N*-diethyldithiocarbamate trihydrate solution (1 in 20), mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake it vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter it if necessary, and use the layer as the test solution.

Separately, to 2.0 mL of the lead standard solution, add water to make exactly 10 mL. To 1.0 mL of this solution, add 10 mL of diammonium hydrogen citrate solution (1 in 4) and 2 drops of bromothymol blue TS, then perform the same procedures as for the test solution, and use the solution so obtained as the standard solution.

Perform the test with the test and standard solutions according to Atomic Absorption Spectroscopy under the following conditions, and determine the concentration of lead in the test solution.

Gas: Dissolved Acetylene or hydrogen gas --- Air

Lamp: Lead hollow-cathode lamp

Wavelength: 283.3 nm

Method 2 Cut a container into pieces smaller than 5 mm square, put 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanone and 0.1 mL of nitric acid, and dissolve it by heating. To this solution, add 96 mL of methanol slowly to precipitate a resinous substance, and filter it by suction. Wash the beaker and the resinous substance with 12 mL of methanol, followed by 12 mL of water, combine the washings and the filtrate, and concentrate to reduced pressure to be about 10 mL, and transfer it to a separatory funnel. Add 10 mL of ethyl acetate and 10 mL of water, shake it vigorously, and allow to stand for a while, allowing the water layer to evaporate to to dryness. Add 5mL of hydrochloric acid to the residue, and dissolve it by heating. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of heated ammonium acetate solution (2 in 5). If there is any insoluble matter, filter it through a glass filter (G3). To this solution, add 10 mL of diammonium hydrogen citrate solution (1 in 4), and 2 drops of bromothymol blue TS, and the add ammonia TS until the color of the solution changes from yellow to green. Add 10 mL of ammonium sulfate solution (2 in 5) and water to make it up to 100 mL. Add 20 mL of sodium *N*,*N*-diethyldithiocarbamate solution (1 in 20) to this solution, mix it well, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake it vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter the layer if necessary, and use the layer as the test solution. Separately, add exactly 5mL of lead standard solution, and add water to make it up to exactly 50 mL, and to 2.0 mL of this solution, add 10 mL of a diammonium hydrogen citrate solution (1 in 4) and 2 drops of bromothymol blue TS, then perform the same procedures as for the test solution, and use it as the standard solution. Perform the test with the test and standard solutions, respectively, according to Atomic Absorption Spectrophotometry under the conditions described in Method 1, and determine the concentration of lead in the test solution.

4) Cadmium

Method 1

To 2.0 mL of cadmium standard solution, add 10 mL of an ammonium citrate solution (1 in 4) and 2 drops of bromothymol blue TS, and perform the same procedures as for the test solution in Method 1, under 3), and use it as the standard solution. Perform the test with the test and standard solutions obtained in Method 1, under 3) according to Atomic Absorption Spectrophotometry under the following conditions and determine the concentration of cadmium in the test solution.

Gas: Combustible gas --- Acetylene or hydrogen Supporting gas --- Air

Lamp: Cadmium hollow-cathode lamp

Wavelength: 228.8 nm

Method 2 To 2.0 mL of cadmium standard solution, add 10 mL of an ammonium citrate solution (1 in 4) and 2 drops of bromothymol blue TS, and perform the same procedures as for

the test solution in Method 2 under 3), and use the solution as the standard solution. Perform the test with the test and standard solutions obtained in Method 2, under 3) according to Atomic Absorption Spectrophotometry under the conditions described in Method 1, and determine the concentration of cadmium in the test solution.

5) Tin

Cut a container into pieces smaller than 5 mm square, place 5.0 g of the pieces in a Kjeldahl flask, add 30 mL of a mixture of sulfuric acid and nitric acid (1 : 1), and decompose it by gently heating in a muffle furnace, occasionally dropwise adding a mixture of sulfuric acid and nitric acid (1 : 1) until the color of the solution changes to a clear, light brown. Then, heat it slowly until the solution becomes a clear, light-yellow concentrate, and heat it again to evaporate to dryness. After cooling it down, add 5 mL of hydrochloric acid to the residue and dissolve it by heating, and after cooling, add water to make exactly 10 mL. Transfer exactly 5 mL of this solution into a 25 mL volumetric flask (A). Transfer the remaining solution to a 25 mL beaker (B) by washing out with 10 mL of water, then add 2 drops of bromocresol green TS, neutralize it with dilute ammonia water (28) (1 in 2), and consider the volume consumed for neutralization as α mL. To volumetric flask A, add potassium permanganate TS dropwise until a slight pale red color develops, and then add a small amount of L-ascorbic acid until it decolorizes. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of citric acid monohydrate solution (1 in 10), α mL of diluted ammonia water (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, and then add 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake it well, then allow it to stand for about 20 minutes, and use it as the test solution. Separately, take exactly 1.0 mL of the tin standard solution, add 5mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, perform the same procedures as for the test solution, and use it as the standard solution. Determine the absorbances of the test and standard solutions at 510 nm, respectively, according to the Ultraviolet-visible Spectrophotometry, using water as the blank test solution.

Eluate testing

Cut the homogeneous regions with low curvature and the same thickness as the container, as far as possible. Gather pieces to make a total surface area of about 1200 cm^2 when the thickness is 0.5 mm or less, or about 600 cm^2 when the thickness is greater than 0.5 mm, and subdivide in general into strips approximately 0.5 cm in width and 5 cm in length. Wash them with water, and dry them at ordinary temperature. Place these strips in a 300 mL hard glass vessel, add exactly 200 mL of water, and properly seal the opening with a suitable stopper. After heating the vessel in an autoclave at 121 °C for 1 hour, take out the vessel, allow to stand until the temperature drops to ordinary temperature, and use it as the test solution. For containers made of composite plastics, the extraction may be performed by filling a labeled volume of water in the container. In this case, it is necessary to record the ratio of extracted liquid volume and the inside area of the container. When containers are deformed at 121 °C, the extraction may be performed at the highest temperature which does not cause deformation. In this case, the relationship between temperature and extraction time is as follows:

for 2 ± 0.2 hours at 100 ± 2 °C
for 24 ± 2 hours at 70 ± 2 °C
for 72 ± 2 hours at 50 ± 2 °C
for 72 ± 1 hours at 37 ± 1 °C

Prepare the blank test solution with water by performing the same procedures. For containers made of composite plastics, water is used as the blank test solution. Perform the following tests with the sample and the blank test solutions.

1) Foaming test

Place 5 mL of the test solution in a glass-stoppered test tube with an internal diameter of about 15 mm and a length of about 200 mm, shake it vigorously for 3 minutes, and measure the time needed for the almost complete disappearance of the foam generated.

2) pH

Take 20 mL each of the sample and the blank test solutions, add 1.0 mL of a potassium chloride solution (1 in 1000) obtained by dissolving 1.0 g of potassium chloride in water to make each to 1000 mL, and calculate the pH difference between these solutions.

3) Potassium permanganate-reducing substances

Put 20.0 mL of the test solution in a glass-stoppered Erlenmeyer flask, add 20.0 mL of 2 mmol/L potassium permanganate solution and 1 mL of dilute sulfuric acid, and boil it for 3 minutes. After cooling, add 0.10 g of potassium iodide, seal tightly with a stopper, shake, then allow to stand for 10 minutes, and titrate it with 0.01 mol/L sodium thiosulfate solution (indicator: 5 drops of starch TS). Perform the blank test separately using the same procedures with 20.0 mL of the blank test solution. Calculate the difference between the amounts of 2 mmol/L potassium permanganate solution used to titrate the sample and blank test solutions.

4) Ultraviolet-visible absorption spectrum

Measure and read the maximum absorbances of the test solution between 220 nm to 240 nm and between 241 nm to 350 nm wavelengths compared to the blank test solution as directed under the Ultraviolet-visible Spectrophotometry.

5) Residue on evaporation

Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying it at 105 °C for 1 hour.

Particulate matter test

Rinse the inside and outside of the container to be used for the tests thoroughly with water, fill the container to the labeled volume of water for the particle matter test or 0.9 w/v % sodium chloride solution, adjust the amount of air in the container to about 50 mL per 500 mL of the labeled volume, seal the container tightly with a stopper, and heat at 121 °C for 25 minutes in an autoclave. After allowing to cool for 2 hours, remove the container from the autoclave, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121 °C, follow regulations on temperature and time under the Eluate testing. Then, rinse the outside of the container, mix the solution by turning upside down 5 – 6 times, and immediately insert a clean needle for filter-less infusion into the rubber stopper of the container, take the effluent in a clean measurement container while shaking it gently, and use it as the test solution.

Test method Measurement of the fine particulate matter is performed in dustless, clean facilities or apparatus using a light-shielded automatic fine particulate matter counter. The sensor of the counter to be used should be able to count fine particulate matter 1.5 μm in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. To calibrate the diameter and the particulate matter count, the standard particulate matters for the calibration of the light-shielded automatic fine

particulate matter counter are suspended in water for fine particulate matter tests or 0.9 w/v % sodium chloride solution, and this solution is used for measurement. Count the numbers of particles with diameters of 5 to 10 μm, 10 to 25 μm, and 25 μm 5 times while stirring the test solution, discard the first measured value, and average the particulate matter count at the other 4 times. This is considered as the particulate matter count in 1.0 mL of the test solution.

Reagent Water for the particulate matter test and 0.9 w/v % sodium chloride solution to be used for the tests should not contain more than 0.5 particulate matter 5 – 10 μm in size per 1.0 mL.

Transparency test

Method 1 This method can only be applied to containers which have a smooth and not embossed surface and rather low curvature. Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces about 0.9 × 4 cm in size, immerse each piece in a cuvette filled with water to determine the ultraviolet-visible absorption spectrum, and determine the transmittance at 450 nm as directed under the Ultraviolet-Visible Spectrophotometry using a cuvette filled with water as the blank.

Method 2 (sensory test) This method can be applied to containers which have a rough or embossed surface. It is applied when testing the transparency of containers of pharmaceutical products in case where the turbidity of their pharmaceutical contents should be checked due to precipitation of pharmaceutical contents.

(i) *Regent, Formazine standard suspension* Dilute 15 mL of formazine stock suspension with water to make 1000 mL. Use it within 24 hours of preparation. Shake thoroughly before use.

(ii) *Reference, Suspension* Dilute 50 mL of formazine standard suspension with water to make 100 mL.

Procedures ① **with control** Take two containers to be tested, and fill one of them with the labeled volume of reference suspension and the other with the same volume of water. Show these 2 containers to 5 subjects, separately, ask which one seems to be more turbid, and calculate the rate of correct answers.

② **without control** Take six containers to be tested, put a number on each of them, and fill three with the labeled volume of the reference suspension and others with the same volume of water. Show each container randomly to five subjects, separately, ask if it is turbid or not, and calculate the percentage who judged it as "turbid" (100X/15: X is the number of containers judged as "turbid") for the reference-filled container group and the water-filled container group, respectively.

Water vapor permeability test

Method 1 This test method is applicable to containers of aqueous injection. Fill the container with the labeled volume of water. After sealing it tightly, accurately weigh the container and record the value. Store the container at 65 ± 5% relative humidity and a temperature of 20 ± 2 °C for 14 days, and then accurately weigh the container again, and to record the value. Calculate the mass loss during storage.

Method 2 This test method is provided for evaluating moisture permeability of the containers for hygroscopic drug. Unless otherwise specified, perform the test using the following procedure.

Desiccant Place a quantity of calcium chloride for water determination in a shallow container, taking care to exclude any fine powder, then dry it at 110 °C for 1 hour, and cool in a desiccator.

Procedure Select 12 containers, wipe their surfaces with a dry cloth, and close and open each container 30 times through the same procedure. Among the containers, 10 containers are used as sample containers and the remaining 2 as control containers. For the screw cap, close each container by applying the torque specified in the table below. Add desiccant to 10 sample containers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or to two-thirds of the capacity if the container volume is less than 20 mL. If the interior depth of the container is more than 63 mm, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and desiccant, but the layer of desiccant in such a container shall be 5 cm in depth. Close each container immediately after adding desiccant by applying the torque designated in the table. Take 2 control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the sample containers, and close it using the same torque as shown above. Record the mass of these individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest 1 mg unit if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at 75 ± 3% relative humidity and a temperature of 20 ± 2 °C. After 14 days, record the mass of the individual containers in the same manner as above. Completely fill 5 empty containers with water or a non-compressible, non-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each container to a graduated glass cylinder, and determine the average volume (mL). Calculate the rate of moisture permeability (mg/day/L) using the following formula:

$$\text{Moisture permeability (mg/day/L)} = (1000/14V)\ \{\ (T_f - T_i) - (C_f - C_i)\ \}$$

V: Average volume (mL)

T_f-T_i: Difference between the final and initial masses of each sample container (mg)

C_f - C_i: Average of difference between the final and initial masses of 2 control containers (mg)

Table. Proper torque for screwed containers

Closure diameter (mm)	Torque (N·cm)
8	59
10	60
13	88
15	59 - 98
18	78 - 118
20	88 - 137
22	98 - 157
24	118 - 206
28	137 - 235
30	147 - 265
33	167 - 284
38	196 - 294
43	196 - 304
48	216 - 343
53	235 - 402
58	265 - 451
63	284 - 490
66	294 - 510
70	314 - 569
83	363 - 735
86	451 - 735
89	451 - 794
100	510 - 794

Closure diameter (mm)	Torque (N·cm)
110	510 - 794
120	618 - 1069
132	677 - 1069

Leakage test

Fill a container with a fluorescein sodium solution (1 in 1000), seal it tightly with the stopper, place filter papers on the top and bottom of the container, and apply a pressure of 6.9 N (0.7 kg)/cm^2 at 20 °C for 10 minutes. Judge the leakiness by observing the color of the paper.

Cytotoxicity test

This test is designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. When preparing a test solution, it is important to take care to prevent contamination by microorganisms and other foreign matters. Other appropriate standard methods of cytotoxicity test may be used for the evaluation, if appropriate. However, the final decision shall be made by this test method if the test results from other methods are questionable.

Cell line The recommended cell line is L929 (ATCC CCL1). This cell line is subcultured in Eagle's minimum essential medium in which fetal calf serum was added. It is cultivated for 24 hours in a carbon dioxide incubator with 5 ± 1% carbon dioxide concentration and 36 °C to 38 °C temperature until the cell layer covers 80% of the plate. Check whether the cell layer is homogeneous and uniform when the cell-culture medium is examined under a microscope. However, other cell lines may be used if the morphology of the cell colonies and the reproducibility of the results are almost same as those of described cell line through the test in advance.

Culture medium Eagle's minimum essential medium shall be used. Dissolve the chemicals listed below in 1000 mL of water. Sterilize the solution by autoclaving it at 121 °C for 20 minutes. Cool the solution to ordinary temperature, and add 22 mL of sterilized sodium bicarbonate solution and 10 mL of sterilized glutamine solution. Add fetal calf serum (FCS) to this solution to be 10 v/v% in the medium.

Sodium chloride	6.80 g
Potassium chloride	400 mg
Sodium dihydrogen phosphate (anhydrous)	115 mg
Magnesium sulfate (anhydrous)	93.5 mg
Calcium chloride (anhydrous)	200 mg
Glucose	1.00 mg
L-arginine hydrochloride	126 mg
L-cysteine hydrochloride (monohydrate)	31.4 mg
L-tyrosine	36.0 mg
L-histidine hydrochloride (monohydrate)	42.0 mg
L-isoleucine	52.0 mg
L-leucine	52.0 mg
L-lysine hydrochloride	73.0 mg
L-methionine	15.0 mg
L-phenylalanine	32.0 mg
L-threonine	48.0 mg
L-tryptophan	10.0 mg
L-valine	46.0 mg
Succinic acid	75.0 mg
Sodium succinate (hexahydrate)	100 mg
Choline bitaetrate	1.8 mg
Folic acid	1.0 mg
Myo-inositol	2.0 mg
Nicotinic acid amide	1.0 mg
D-calcium pantothenate	1.0 mg
Pyridoxal hydrochloride	1.0 mg
Riboflavin	0.1 mg
Thiamine hydrochloride	1.0 mg
Biotim	0.02 mg
Phenol red	6.0 mg

Reagent

Sodium bicarbonate solution Dissolve 10 g of sodium bicarbonate in water to make 100 mL. Sterilize the solution either by autoclaving in a tight container at 121 °C for 20 minutes or by filtering it through a membrane filter with a nominal pore diameter of 0.22 μm.

Glutamine solution Dissolve 2.92 g of L-glutamine in water to make 100 mL. Sterilize the solution by filtering it through a membrane filter with a pore diameter of 0.22 μm.

Phosphate buffer solution Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogen phosphate, 8.00 g of sodium chloride, and 1.15 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL. Sterilize the solution by autoclaving it at 121 °C for 20 minutes.

Trypsin solution Dissolve 0.5 g of trypsin and 0.2 g of ethylenediaminetetraacetic acid disodium in phosphate buffer solution to make 1000 mL. Sterilize the solution by filtering it through a membrane filter with a pore diameter of 0.22 μm.

Dilute formaldehyde TS Perform a 10-fold dilution of formaldehyde solution by adding water.

Dilute giemsa staining TS Dilute a commercially available Giemsa staining solution by a factor of fifty with the diluent. Prepare it before use.

Diluent Dissolve 4.54 g of potassium dihydrogen phosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL.

Devices and apparatus

Pipet Pasteur pipet, measuring pipet, and pipet with microtip.

Screw-capped glass bottle 50 to 1000 mL.

Sterile disposable plastic centrifuge tube 15 and 50 mL

Sterile disposable plastic tissue culture flask 25 cm^2 or 75 cm^2

Sterile disposable plastic tissue culture plate (24 wells).

Microscope Inverted microscope and stereoscopic microscope.

Carbon dioxide incubator Maintains a temperature of 37 °C and a CO_2 gas concentration of 5%

Control materials

Negative control material Polyethylene film

Positive control material A Polyurethane film containing 0.1% zinc diethyldithiocarbamate.

Positive control material B Polyurethane film containing 0.25% zinc dibutyldithiocarbamate.

Control materials Zinc diethyldithiocarbamate and zinc dibutyldithiocarbamate (Reagent Grade 1).

Sample Preparation If the container material is homogeneous layer, subdivide the cut pieces of a container into pieces with a size of approximately 2 × 15 mm and use them as the sample for the test. If the container material has multiple layers, prepare cut pieces with a surface area of one side of 2.5 cm^2 from the container and use it as the sample for the test, without subdividing them into smaller pieces.

Preparation of test solution Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable plastic centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle

or tube by autoclaving at 121 °C for 20 minutes. If the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used under certain conditions. Sufficient aeration should be achieved to prevent an effect of residual EO. To the bottle or tube, add the culture medium in a proportion of 1 mL per 2.5 cm^2 (one side surface) of the sample surface or 10 mL per 1 g of the sample, loosely cap the bottle or tube, and allow to stand in an incubator, maintaining 5% carbon dioxide at 37 °C for 24 hours. Transfer extract, which is designated 100% test solution, to a sterilized screw-capped glass bottle or sterile disposable centrifuge tube. Dilute the test solution serially by using fresh culture medium with a dilution factor of two to prepare test solutions with concentrations of 50%, 25%, 12.5%, 6.25%, and 3.13% and so on.

Preparation of cell suspension Remove the culture medium from the sterile tissue culture flask where cells are cultured, and wash the cells with an appropriate volume of phosphate buffer solution by gently tilting the flask 2 – 3 times, then discard the PBS. Add a sufficient volume of trypsin TS to cover the cell layer. Cap the flask and place the flask in a carbon dioxide incubator for 1 – 2 minutes. Remove the flask from the incubator, and observe the detachment of the cell layer under a microscope. Add an appropriate volume of the fresh culture medium and gently pipette the cells completely out of the flask with a Pasteur pipette. Transfer the pipetted cell suspension into a sterile disposable plastic centrifuge tube and centrifuge the tube at 800 – 1000 rpm for 2 – 5 minutes. Discard the clear solution from the supernatant, add an appropriate volume of PBS to pipette the solution with a Pasteur pipette, and then centrifuge it again. Discard the clear solution from the supernatant, and add an appropriate volume of fresh culture medium. Resuspend the cells by pipetting the solution with a Pasteur pipette to make a homogeneous cell suspension. Determine the cell concentration using a hemocytometer.

Cytotoxicity testing Dilute the cell suspension with culture medium to adjust the cell concentration to 100 cells/mL. Distribute 0.5 mL aliquot of the dilute cell suspension on each well of a sterile disposable multiple well plate. Perform stationary incubation of the culture plate in the carbon dioxide incubator for 4 – 6 hours to make the cells attach to the bottom surface of the plate. Discard the medium in each well of the plate and add 0.5 mL of the test solution prepared before with several different concentrations or new medium to each well. Each test solution of each concentration or new medium is loaded into 4 wells, respectively. Immediately place the plate in the carbon dioxide incubator again and incubate it for the specified period. The incubation period for L929 cells is 7 to 9 days. After the incubation, discard the test solution in the culture plate, add an appropriate volume of dilute formaldehyde TS to each well and leave the plate for 30 minutes to fix the cells. Discard the dilute formaldehyde TS in each well and add an appropriate volume of dilute Giemsa staining TS to each well. After ensuring that the colonies were stained well, discard the dilute Giemsa staining TS in the wells and count the number of colonies in each well. Calculate a mean number of colonies in the test solution with each concentration and divide the value by the mean number of colonies in the medium only to obtain the colony formation rate (%) for each concentration of the test solution. Plot the concentration (%) of the test solution on a logarithmic axis and the colony formation rate on the other axis on semi-logarithmic graph paper to obtain a colony formation inhibition curve for the container. Read the percent concentration the test solution which inhibits colony formation to 50% (IC_{50} (%)) from the curve.

If necessary, check the sensitivity and the reproducibility of the test system by testing with suitable control materials or control substances.

Plastic container for aqueous injections

This is a plastic container used for aqueous injections. Plastic containers for aqueous injections should not interact with the pharmaceuticals contained therein to alter their efficacy, safety or stability, and should not permit contamination with microorganisms. The containers also meet the following requirements.

1) Polyethylene or polypropylene containers for aqueous injections

The containers are made of polyethylene or poly-propylene and free from any adhesive.

A) Transparency

The containers have a transmittance of NLT 55%, when tested as directed in Method 1 of Transparency Test. When Method 1 cannot be applied, ② No-control Test of Method 2 of Transparency Test is used. In this case, the rate at which the water-containing containers are judged as "being turbid" is less than 20%. And the rate at which the reference suspension-containing containers are judged as "being turbid" is NLT 80%.

B) Appearance

The containers should not have strips, cracks, bubbles, or other damages which cause difficulties in practical use.

C) Water vapor permeability

The loss of mass in Method 1 is 0.20%.

D) Heavy metals

The color of the test solution is not darker than that of the control solution. The amount of the pieces taken from the container is 1.0g.

E) Lead

The absorbance of the test solution is NMT that of the standard solution in the test according to Method 1.

F) Cadmium

The absorbance of the test solution is NMT that of the standard solution in the test according to Method 1.

G) Residue on ignition

The residue is NMT 0.10% (5 g).

H) Eluate

① **Foaming test** The foam formed almost completely disappears within 3 minutes.

② **pH** The difference in pH between the test solution and the blank test solution is NMT 1.5.

③ **Potassium permanganate-reducing substances** The difference in the consumption of 0.002 mol/L potassium permanganate solution between the test solution and the blank test solution is NMT 1.0 mL.

④ **Ultraviolet-visible absorption spectrum** The maximum absorbance between 220 nm and 240 nm is NMT 0.08, and that between 241 nm and 350 nm is NMT 0.05.

⑤ **Residue on evaporation** NMT 1.0 mg.

I) Cytotoxicity

IC_{50}(%) is NMT 90%. The results obtained by the other standard methods are negative.

2) Other containers for aqueous injections

The other containers meet the following specifications and other necessary specifications for their materials with regard to heavy metals, residue on ignition, eluate, etc.

A) Transparency

Proceed as directed in A) under Polyethylene or Polypropylene Containers for Aqueous Injections.

B) Appearance

Proceed as directed in B) under Polyethylene or Polypropylene Containers for Aqueous Injections.

C) Water vapor permeability

Proceed as directed in C) under Polyethylene or Polypropylene Containers for Aqueous Injections.

D) Cytotoxicity

Proceed as directed in I) under Polyethylene or Polypropylene Containers for Aqueous Injections.

Polyacrylamide Gel Electrophoresis
폴리아크릴아미드겔 전기영동법

1. Principal

Electrophoresis uses the principle that an electrically charged matter moves to an electrode with an opposite electric charge under an electric field. In gel electrophoresis, the migration of particles is interfered with by the interaction with the gel matrix that acts as the molecular filtration. The migration speed in the gel depends on the size, shape and type of charge of the particles due to the opposing action of molecular filtration by electric force. Due to the difference in these physicochemical properties, macromolecules in the mixture move at different speeds and are separated during electrophoresis.

Scope

Polyacrylamide gel electrophoresis (PAGE) is used for the qualitative analysis of protein characteristics in biological preparations, as well as for purity test, and for quantitative analysis.

Purpose

Analytical gel electrophoresis is an appropriate method with which to identify and to assess the homogeneity of proteins in pharmaceutical preparations. The method is routinely used to measure the molecular weights of protein subunits and for determination of the subunit compositions of purified proteins. Ready-to-use gels and reagents are commercially available and can be used instead of those described in this text, provided that they give equivalent results and that they meet the validity requirements.

1.1. Characteristics of polyacrylamide gel

The filtration properties of polyacrylamide gel stem from the formation of a three-dimensional fibrous network when bifunctional bisacrylamide crosslinks with polyacrylamide chains, creating pores. This polymerization is catalyzed by ammonium persulfate and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED).

As the acrylamide concentration in the gel increases, the pore size decreases. The effective pore size of a gel is operationally defined by its filtration properties, that is, by the resistance it imparts to the migration of macromolecules. The available concentration of acrylamide has limitations because high concentration of gel makes the gel brittle and difficult to handle, resulting in a smaller pore size, which slows down the rate at which proteins can move through the gel. Therefore, the pore size of gel should be determined by adjusting the acrylamide concentration properly to ensure that the separation of the protein to be analyzed is optimized. Changes in acrylamide and bisacrylamide composition reflect the physical properties of the gel used.

In addition to the composition of the gel, the condition of protein is also an important factor in protein migration in electrophoresis. The migration of proteins on electrophoresis is determined by the dissociation constants (pK) and the molecular sizes of the charged groups. It is also affected by the type, concentration, pH, and temperature of the buffer solution, strength of electric field, and characteristics of the support.

2. Denaturing polyacrylamide gel electrophoresis (PAGE)

This method is mainly used for the analysis of proteins with molecular weights in the range of 14,000 to 100,000 Da. However, various techniques such as gradient gels or specific buffer solution systems can be used for broader range of molecular weight. For instance, tricine–sodium dodecyl sulfate (SDS) gels, using tricine as the trailing ion in the electrophoresis running buffer solution, can separate very small proteins and peptides under 10,000 – 15,000 Da.

Denaturing Polyacrylamide Gel Electrophoresis (PAGE) using glycine SDS is the most commonly employed electrophoresis method for assessing the pharmaceutical quality of protein.

products. The denatured proteins acquire a negative charge by binding with sodium dodecyl sulfate, exhibiting a constant charge-to-mass ratio irrespective of the protein's shape. As the amount of sodium dodecyl sulfate bound to a protein is proportional to the molecular weight of the protein and independent of the amino acid sequences, the sodium dodecyl sulfate-protein complex migrates according to the size of the proteins on the polyacrylamide gel.

The electrophoretic mobilities of the resultant detergent–polypeptide complexes all assume the same functional relationship to their molecular masses. In electrophoresis, it is assumed that the migration of sodium dodecyl sulfate-protein complexes is inversely proportional to their molecular weight. Therefore, smaller sodium dodecyl sulfate-protein complexes migrate to the cathode faster than the larger ones. Accordingly, the molecular weight of each protein can be measured from the relative distance of its migration in the polyacrylamide gel for calibration, and the purity can be measured with each separated protein band.

However, if the protein is *N*- or *O*-glycosylated, it has a significant effect on the measurement of protein molecular weight. This is because sodium dodecyl sulfate is not bound to the glycosylated site of the glycoprotein at a constant charge-to-mass ratio. Therefore, the molecular weight of the post-translational modified protein does not always match the molecular weight of the polypeptide chain.

2.1 Non-reducing condition

In some cases, it is not appropriate to analyze proteins under reducing conditions. Polypeptide subunits and three-dimensional structure often are maintained in proteins by the presence of disulfide bonds. A goal of SDS-PAGE analysis under reducing conditions is to disrupt this structure by reducing disulfide bonds.

When treated with 2-mercaptoethanol or dithiothreitol (DTT) reducing agent, proteins bind to sodium dodecyl sulfate as its tertiary structure is unfolded. Under these conditions, molecular weights of the proteins can be calculated by applying linear regression analysis in the presence of the reference standard with a proper molecular weight.

2.2 Non-reducing condition

In some cases, it is not proper to analyze proteins under reducing conditions. If not treated with the reducing agents such as 2-mercaptoethanol or dithiothreitol, the oligomeric form of the protein is maintained since its disulfide bond is preserved. Oligomeric disulfide protein complexes migrate more slowly than their

SDS–polypeptide subunits. Moreover, as non-reducing proteins are not completely saturated with sodium dodecyl sulfate, sodium dodecyl sulfate does not bind to the proteins at a constant mass ratio.

The analysis of protein molecular weights may be less accurate than those from assays under reducing conditions because molecular weight reference standards and samples should have similar structures for effective analysis. Nevertheless, the purity of the protein to be analyzed can be measured using the bands separated in the gel under these conditions.

3. Electrophoresis of discontinuous buffer solution system

The electrophoresis of a discontinuous buffer solution system, composed of two adjacent and different gels -the upper resolving gel and the lower separating gel, is commonly employed in the analysis of protein mixtures. The two gels exhibit distinct characteristics, such as varying pore sizes, pH and ionic strengths. Additionally, different mobile ions are used in both gels and electrophoresis buffer solutions. Such this discontinuity of buffer solutions increases the degree of separation by concentrating a large volume of sample into the resolving gel. If electricity is applied, a voltage drop is formed between the samples, and the proteins migrate to the resolving gel, followed by the glycine salt in the electrophoresis buffer solution to the resolving gel. A migration boundary region swiftly forms between chloride ions, which move rapidly to the front, and glycine salt ions, which move relatively slowly to the back. A localized high-voltage gradient forms between the leading and trailing ion fronts, causing the sodium dodecyl sulfate-protein complexes to form into a thin zone (stack) and to migrate between the chloride and glycinate phases. As a result, sodium dodecyl sulfate-protein complexes form a thin zone, migrating between the chloride and glycinate phases.. Most of the resolving gels with large pores do not hinder the migration of proteins and play a role in preventing the spread of proteins. At the interface between the resolving gel and the separating gel, the migration of proteins is greatly hindered due to the limited pore size of the separating gel and the buffer discontinuity. Once migrated into the resolving gel, the migration of the proteins is further slowed down by the relatively small pore size of the gel medium. However, the glycine salt ion migrates faster than proteins in the homogeneous pH region formed by trishydroxymethylaminomethane and glycine salt. Separating gels facilitate the separation of proteins based on their molecular weights.

4. Preparation of discontinuous vertical buffered sodium dodecyl sulfate-polyacrylamide gel

This section describes the preparation of gels using particular instrumentation. This does not apply to precast gels. For precast gels or any other commercially available equipment, the manufacturer's instructions must be used for guidance. The use of commercial reagents that have been purified in solution is recommended. When this is not the case and when the purity of the reagents used is not sufficient, a pretreatment is applied. For instance, any solution sufficiently impure to require filtration must also be deionized with a mixed-bed (anion–cation exchange) resin to remove acrylic acid and other charged degradation products. When stored according to recommendations, acrylamide/bisacrylamide solutions and solid persulfate are stable for extended periods.

4.1 Preparation of gel plate

Clean two glass plates (e.g., 10 cm × 8 cm), the polytetrafluoroethylene comb, two spacers, and the silicone rubber tube (e.g., 0.6 mm × 35 cm) with a mild detergent. Rinse thoroughly with water and ethanol, then dry the tray at ordinary temperature.

Spacers along each of the two short sides of the glass plate, 2 mm away from the edges and 2 mm away from the long side corresponding to the bottom of the gel. Place the tubing on the glass plate by using one spacer as a guide. Twist the tubing at the bottom of the spacer, following the long side of the glass plate. While holding the tubing with one finger along the long side, twist the tubing again and place it on the second short side of the glass plate, using the spacer as a guide. Place the second glass plate in perfect alignment, and hold the mold together by hand pressure. Apply two clamps on each of the two short sides of the mold. Carefully apply four clamps on the longer side of the gel mold, thus forming the bottom of the gel mold. Check that the tubing is placed along the edge of the glass plate and does not protrude while tightening the clamps. Fill the gel mold formed this way with the gel.

4.2 Gel preparation

In the discontinuous buffered sodium dodecyl sulfate-polyacrylamide gel, it is necessary to pour and solidify the resolving gel first before pouring the stacking gel as the composition of acrylamide-bisacrylamide, buffer solution, and pH of the resolving gel are different from those of the stacking gel.

4.2.1 Preparation of the resolving gel

Prepare the required amount of acrylamide solution at the desired concentration in an Erlenmeyer flask as shown in Table 1. Mix the ingredients in the given order. If necessary, before adding the *Ammonium Persulfate Solution* and the *TEMED*, filter the solution under vacuum through a cellulose acetate membrane (pore diameter: 0.45 µm). Keep the solution under vacuum, while swirling the filtration unit, until no more bubbles are formed in the solution. As shown in Table 1, add the proper amount of ammonium persulfate solution and TEMED, shake it, and immediately pour it into the space between the prepared gel plates. At this time, leave enough space for the stacking gel, including about 1 cm of the length of the comb teeth. Thereafter, carefully pour isobutanol saturated with water over the resolving gel solution, and let the gel polymerized at ordinary temperature.

4.2.2 preparation of the stacking gel

After polymerization is complete (about 30 minutes), pour off the isobutanol, discard the isobutanol and rinse the top of the gel with water several times to remove the isobutanol overlay and any unpolymerized acrylamide. After thorough washing, use a paper towel to remove any remaining solution on the stacking gel.

Prepare the required amount of acrylamide solution at the desired concentration in an Erlenmeyer flask as shown in Table 2. Where appropriate, before adding the *Ammonium Persulfate Solution* and the *TEMED*, filter the solution if necessary under vacuum through a cellulose acetate membrane (pore diameter: 0.45 µm). Keep the solution under vacuum, while swirling the filtration unit, until no more bubbles are formed in the solution. Add appropriate amounts of *Ammonium Persulfate Solution* and *TEMED*, as indicated in *Table 2*, and insert a clean comb carefully while taking precautions not to make any air bubbles in the stacking gel solution. Add more stacking gel solution to fill the spaces of the comb completely. Place the gel vertically at ordinary temperature for polymerization.

4.3 Preparation of the sample

Unless otherwise specified in the monograph, prepare samples as follows:

Sample Buffer Solution 1 **(non-reducing conditions)**

Dissolve 1.89 g of trishydroxymethylaminomethane, 5.0 g of sodium dodecyl sulfate, 50 mg of bromophenol blue, and 25.0 mL of glycerin in 100 mL of water. Adjust the pH to 6.8 with hydrochloric acid, add water to make the final volume of 125 mL, mix it well and store. Mix Sample Buffer Solution 1 with the sample in a 1:1 ratio before use.

Sample Buffer Solution 2 **(reducing conditions)** Dissolve 3.78 g of trishydroxymethylaminomethane, 100 g of sodium dodecyl sulfate, 100 mg of bromophenol blue, and 50.0 mL of glycerin in 100 mL of water. Add 25.0 mL of 2-mercaptoethanol, a reducing reagent. Adjust the pH to 6.8 with hydrochloric acid, add water to make the final volume of 250.0 mL, mix it well and store it. If dithiothreitol, another reducing reagent, should be used instead of 2-mercaptoethanol, dissolve 3.78 g of trishydroxymethylaminomethane, 10.0 g of sodium dodecyl sulfate, 100 mg of bromophenol blue, and 50.0 mL of glycerin in 200 mL of water. Adjust the pH to 6.8 with hydrochloric acid, and add additional water to make the final volume of 250.0 mL. Add dithiothreitol before use to achieve a final concentration of 100 mmol/L.

Electrophoresis Buffer Solution Dissolve 151.4 g of trishydroxymethylaminomethane, 721.0 g of glycine, and 50.0 g of sodium dodecyl sulfate in water, add water to make 5 L of 10-fold stock solution of electrophoresis buffer solution. Dilute this stock solution 10 times with water and adjust the pH to 8.1 through 8.8 before use.

Preparation of the sample (non-reducing conditions): Mix equal volumes of water plus the sample or reference solutions and Sample Buffer (Concentrated).

Preparation of the sample (reducing conditions): Mix equal volumes of water plus the sample or reference solutions and Sample Buffer for Reducing Conditions (Concentrated) containing 2-ME (or DTT) as the reducing agent. The concentration can vary based on protein and staining methods.

Sample treatment: Place in a boiling water bath or a block heater set at 100 °C for 5 minutes and then cool. (Note that temperature and time may vary in the monograph because protein cleavage may occur during the heat treatment.).

4.4 Mounting the gel in the electrophoresis apparatus and electrophoretic separation

Once the stacking gel is solidified enough after polymerization (about 30 minutes), carefully remove the polytetrafluoroethylene comb inserted to generate gaps in the stacking gel for the sample to be loaded. Rinse these gaps immediately with water or electrophoresis buffer to eliminate any remaining unpolymerized acrylamide. If necessary, tidy up the gaps with a needle. Carefully mount the gel plate on the electrophoresis apparatus.

Remove the clamps on one short side, gently pull out the tubing, and replace the clamps. Repeat this process on the other short side. Disconnect the tubing from the bottom part of the gel.

Table 1. Resolving gel preparation

	Volume of composition (mL) / Gel volume							
Composition of solution	**5 mL**	**10 mL**	**15 mL**	**20 mL**	**25 mL**	**30 mL**	**40 mL**	**50 mL**
6% polyacrylamide gel								
Water	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
Acrylamide solution [(1)]	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED [(5)]	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% polyacrylamide								
Water	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
Acrylamide solution [(1)]	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.3	2.5	3.8	5.0	6.3	6.3	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
TEMED [(5)]	0.003	0.006	0.006	0.012	0.015	0.018	0.024	0.03
10% polyacrylamide gel								
Water	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
Acrylamide solution [(1)]	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.3	2.5	3.8	5.0	6.3	6.3	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
TEMED [(5)]	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% polyacrylamide gel								
Water	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
Acrylamide solution [(1)]	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.3	2.5	3.8	5.0	6.3	6.3	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
TEMED [(5)]	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
14% polyacrylamide gel								
Water	1.4	2.7	3.9	5.3	6.0	8.0	10.6	13.8
Acrylamide solution [(1)]	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.2	2.5	3.6	5.0	6.3	6.3	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
TEMED [(5)]	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% polyacrylamide gel								
Water	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5

	Volume of composition (mL) / Gel volume							
Composition of solution	**5 mL**	**10 mL**	**15 mL**	**20 mL**	**25 mL**	**30 mL**	**40 mL**	**50 mL**
Acrylamide solution [(1)]	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 mol/L Tris buffer (pH 8.8) [(2)]	1.3	2.5	3.8	5.0	6.3	6.3	10.0	12.5
SDS solution [(3)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
Ammonium persulfate solution [(4)]	0.05	0.1	0.15	0.2	0.25	0.25	0.4	0.5
TEMED [(5)]	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

(1) **Acrylamide solution**: Prepare a solution dissolving 290 g of acrylamide and 10 g of methylenebisacrylamide dissolved in 1 L of warm water and filter. [During storage, acrylamide and methylene bisacrylamide transform into acrylic acid and bisacrylic acid, respectively. As such deamination is catalyzed by light and alkali, the pH of this solution should be 7.0 or lower, and it should be stored in a light-resistant container and refrigerated. Do not store it for a long period of time.]

(2) **1.5 mol/L Tris buffer (pH 8.8)**: Add about 90.8 g of tris(hydroxymethyl)aminomethane to a 500-mL flask and dissolve in 400 mL of water. Adjust the pH to 8.8 with hydrochloric acid and then add water to make 500 mL.

(3) **SDS solution**: Prepare a solution with 100 g of sodium dodecyl sulfate (suitable for electrophoresis) per 1 L of water and store it at ordinary temperature.

(4) **Ammonium persulfate solution**: Prepare a small amount of a solution containing 100 g of ammonium persulfate per 1 L of water and store at 4 °C. [Ammonium persulfate provides free radicals that polymerize acrylamide and methylene bisacrylamide and does not have a long shelf life because it decomposes quickly.]

(5) **N,N,N',N'-tetramethylethylenediamine (TEMED)**: Use a solution suitable for electrophoresis. [TEMED catalyzes the formation of free radicals from ammonium persulfate and promotes the polymerization of acrylamide and methylene bisacrylamide. As TEMED acts only as a free base, polymerization is inhibited at low pH.]

Table 2. Stacking gel preparation

	Volume of Composition (mL) / Gel Volume							
Composition of solution	**1 mL**	**2 mL**	**3 mL**	**4 mL**	**5 mL**	**6 mL**	**8 mL**	**10 mL**
Water	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
Acrylamide solution [(1)]	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.5 mol/L Tris buffer (pH 8.8) [(2)]	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
SDS solution [(3)]	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
Ammonium persulfate solution [(4)]	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED [(5)]	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

(1) **Acrylamide solution**: Prepare a solution containing 290 g of acrylamide and 10 g of methylenebisacrylamide dissolved in 1 L of warm water and filter.

(2) **1.5 mol/L Tris buffer (pH 8.8)**: Add about 90.8 g of tris(hydroxymethyl)aminomethane to a 500-mL flask and dissolve in 400 mL of water. Adjust the pH to 8.8 with hydrochloric acid and then add water to make 500 mL.

(3) **SDS solution**: Prepare a solution containing 100 g of sodium dodecyl sulfate (suitable for electrophoresis) per 1 L of water and store at ordinary temperature.

(4) **Ammonium persulfate solution**: Prepare a small amount of a solution containing 100 g of ammonium persulfate per 1 L of water and store at 4 °C. [Ammonium persulfate provides free radicals that polymerize acrylamide and methylene bisacrylamide and does not have a long shelf life because it decomposes quickly.]

(5) **N,N,N',N'-tetramethylethylenediamine (TEMED)**: Use a solution suitable for electrophoresis.

Place the gel on the electrophoresis apparatus. Fill the upper and lower tanks with electrophoresis buffer. Remove air bubbles between the stacking gel with a bent needle. Since the buffering effect of the stacking gel may be lost, do not perform preliminary electrophoresis before loading the sample into the stacking gel. Before loading the sample, carefully rinse the stacking gel with electrophoresis buffer.

Prepare the test solution by mixing the sample and the buffer in a 1:1 ratio, following the method specified in the reagent preparation section. Load the proper amount of the sample and reference standard into the stacking gel. As there is a difference in apparatus usage method by electrophoresis manufacturer, conduct the electrophoresis for the test purpose by adjusting the conditions according to the method suggested by the manufacturer. Optimal conditions for protein separation may differ from those suggested by the manufacturer in terms of time and voltage when conducting the electrophoresis. Real-time progress of electrophoresis can be recognized through the migration state of the dye. At this time, check whether the dye migrates to the resolving gel. Stop the electrophoresis when the dye reaches the bottom of the gel. Remove the gel from the electrophoresis apparatus, cut off the stacking gel, and proceed immediately with protein staining.

4.5 SDS PAGE – Gradient concentration gels

Prepare gradient gels with increasing acrylamide concentration from the top to the bottom. Utilize a gradient-forming apparatus for preparation, or use ready-to-use gradient gels with recommended protocols available commercially.

Gradient gels offer some advantages over fixed-concentration gels. Some proteins that co-migrate on fixed-concentration gels can be resolved within gradient gels. During electrophoresis the proteins migrate until the pore size stops further progress, and therefore a stacking effect occurs, resulting in sharper bands. According to Table 3, gradient gels also allow separation of a wider range of protein molecular masses than do single, fixed-concentration gels.

Table 3 suggests compositions for linear gradients, correlating acrylamide concentrations with appropriate protein molecular ranges. Note that other gradient shapes (e.g., concave) can be prepared for specific applications.

Table 3

Acrylamide (%)	Protein range (kDa)
5 – 15	20 – 250
5 – 20	10 – 200
10 – 20	10 – 150
8 – 20	8 – 150

Gradient gels also are used for molecular mass determination and protein purity determination.

4.6 Detection of proteins in gels

Coomassie and silver staining are the most common protein staining methods and are described in more detail below. Additional commercial stains, detection methods, and kits are available, such as fluorescent stains visualized with a fluorescent imager, offering a linear response across a broad range of protein concentrations, often spanning several orders of magnitude, dependent on the protein.

Coomassie staining has a protein detection level of approximately 1–10 μg of protein per band. Silver staining is the most sensitive method for staining proteins in gels, and a band containing 10–100 ng can be detected. These figures are considered reliable for these gel contexts, with reported improvements in sensitivity by one or two orders of magnitude in the literature.

Coomassie staining responds in a more linear manner than silver staining, but the response and range depend on the protein and development time. Both methods may show less reproducibility if the staining process is subjectively stopped, i.e., when the analyst deems the staining satisfactory. Wide dynamic ranges of reference proteins are important to use because they help assess the intra-experimental sensitivity and linearity. All gel-staining steps should be conducted wearing gloves, at ordinary temperature, with gentle shaking (e.g., on an orbital shaker platform), and using any convenient container.

Reagent

Coomassie staining solution: Dissolve 1.25 g of acid blue 83 in 1 L of the mixture of water, methanol, and ultra-high purity acetic acid (5 : 4 : 1), filter through a filter paper, and store at ordinary temperature.

Destaining solution: Mixture of water, methanol, and ultra-high purity acetic acid (5 : 4 : 1)

Fixing solution I: Mixture of water, methanol, and trichloroacetic acid (5 : 4 : 1)

Fixing solution II: Put 250 mL of methanol in a 500 mL flask, add 0.27 mL of formaldehyde, and dilute it with water to make 500 mL.

Silver nitrate solution: Add 8 mL of 200 g/L silver nitrate solution dropwise to 40 mL of a mixture of 40 mL of 1 mol/L sodium hydroxide solution and 3 mL of ammonia water while stirring, and then add water to make 200 mL.

Developing solution: Dilute 2.5 mL of 2% citric acid solution and 0.27 mL of formaldehyde with water to make 500 mL.

Blocking solution: 10% acetic acid

4.6.1 Coomassie staining

Immerse the gel in enough Coomassie Staining Solution to fully submerge it. After stirring for at least 1 hour, remove the solution and slowly destain the gel with a stirrer and Destaining Solution. Then, slowly destain the gel in a stirrer with a sufficient amount of destaining Solution. Destain the gel by changing the Destaining Solution several times until the stained protein band is clearly visible against the clear background. If the gel is destained properly, the detection sensitivity is increased and even a small amount of protein can be identified. If a small amount of anion exchange resin or a small sponge is put into Destaining Solution, destaining process can be expedited. As the acid-alcohol solutions used don't completely fix proteins in the gel, treating with Fixing Solution I for about 1 hour before Coomassie Staining prevents loss of low-molecular-weight proteins during destaining.

4.6.2 Silver staining

Prepare Fixing Solution II in an amount sufficient to fully submerge the gel, stir it slowly for about 1 hour, remove Fixing Solution II, add new Fixing Solution II, and incubate it for at least 1 hour or overnight. Discard Fixing Solution II and stir the gel with plenty of water for 1 hour. After soaking in a 1% (v/v) glutaraldehyde solution for 15 minutes, rinse twice with water for 15 minutes, respectively. Then soak in the nitrate solution for 15 minutes and rinse three times with water for 5 minutes, respectively. Silver Nitrate Solution should be freshly prepared each time an experiment is conducted in the darkroom. Developing the gel in Developing Solution for about 1 minute, stir in fixative for 15 minutes to prevent excessive development, then rinse with water.

4.7 Recording the results

Gels are photographed or scanned while they are still wet or after an appropriate drying procedure. Currently, gel-scanning systems with data analysis software are commercially available to photograph and analyze the wet gel immediately.

The different drying methods are used for gel drying depending on the staining methods. For Coomassie staining, incubate the gel in the 100 g/L glycerin solution for at least 2 hours after destaining. For silver staining, incubate the gel in the 100 g/L glycerin solution for at least 2 hours, and then in the 20 g/L glycerin solution for 5 minutes as the rinse step.

Drying between cellulose films may result in cracking, so carefully assemble and dry cellulose films to avoid this.

After soaking two pieces of porous cellulose films in water for 5 to 10 minutes, place one of them on a drying rack, carefully lift the gel, and place it on the cellulose film. After removing the air bubbles trapped, pour the proper amount of water around the outside of the gel not to form air bubbles. Put the second cellulose film on top and remove any trapped air bubbles. After assembling the drying frame, leave it in an oven or at ordinary temperature until dry.

4.8 Determination of molecular weight

Determining the molecular weight of a protein involves comparing the migration distance of indicator proteins with known molecular weights. Indicator proteins used should be mixtures of proteins whose molecular weights are known and staining of them should be homogeneous. Start by diluting the concentrated stock solution of indicator proteins, which contains proteins with a wide range of molecular weights, in the appropriate sample buffer. Load this mixture onto the gel alongside the sample.

Immediately after the gel has been run, mark the position of the bromophenol blue tracking dye to identify the leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front.

After staining, measure the migration distance of the protein band (indicator or sample protein) from the top of resolving gel, check the position of the bromophenol blue dye and divide its migration distance by the bromophenol blue migration distance. The normalized migration distance calculated in this way is called the relative mobility of the protein relative to the bromophenol blue dye and is referred to as the Rf value. A logarithmic graph of the molecular weight of the indicator protein (MR) compared to the Rf value is generated, which may be slightly sigmoidal. If the value of unknown protein is located on the linear line of the graph, the unknown molecular weight can be calculated by linear regression analysis (more accurately, by nonlinear regression analysis) from the log MR curve for Rf.

4.9 Test validation

The test is considered invalid if the target resolution range of the gel has not been demonstrated. For instance, if the smallest protein in the molecular weight indicator proteins migrates within 80% of the total length of the gel, etc., or if the drug protein and dimer of the drug protein or impurities derived from the drug protein are not separated from each other. When each protein is separated from each other, there is a linear relationship between R_f and log molecular weight. If the plot has a sigmoidal shape, then only data from the linear region of the curve can be used in the calculations. Additional validation requirements are specified in the monograph.

Sensitivity also must be validated. A reference protein control corresponding to the desired concentration limit, run alongside samples, serves as a system suitability check.

4.10 Quantification of impurities

SDS-PAGE is often used as a limit test for impurities. When quantifying impurities by normalizing to the main band using an integrating densitometer or image analysis, the responses must be validated for linearity. Note that depending on the detection method and protein, as described in the introduction of the section 4.6 Detection of Proteins in Gels. Evaluate this range within each run by using one or more control samples with an appropriate range of protein concentrations.

If the limit of impurity protein is specified in the monograph, the test solution should be diluted to make the standard solution for measuring the limit of impurity protein. For example, if the standard of the impurity protein content is 5% of the drug protein content, dilute the drug protein test solution with the diluent at a 1:20 ratio. After electrophoresis, any impurity band in the test solution, except for the main band, should not be larger than the band obtained in the standard solution of 5% content.

Under validated conditions, impurities can be quantified by normalization to the main band, using an integrating densitometer, or by image analysis.

Pyrogen
발열성물질시험법

The Pyrogen is a method to test the existence of pyrogens using rabbits.

Test animals

Use healthy rabbits weighing NLT 1.5 kg, which have not lost their weights when they are raised on a regular diet for at least 1 week before use, are used as test animals. Place the rabbits individually in a stimulus-free environment to avoid excitement. Maintain the ordinary temperature within the range of 20 °C and 27 °C for at least 48 hours before and during the test.

For rabbits that are used for the test for the first time, acclimatize it 1 to 3 days before the test by a placebo test that includes all procedures except injection. If the rabbit has already been used for the test, let it rest for at least 48 hours. However, rabbits that have been administered a sample that was adjudged pyrogen-positive or that contained an antigen present commonly in the test specimen to be examined should not be reused.

Apparatus

1) Thermometer

Use a rectal thermometer or body temperature-measuring device with an accuracy of ± 0.1 °C or less.

2) Syringes and injection needles

Ensure the syringes and needles are free from pyrogens by heating at 250 °C for NLT 30 minutes in a hot-air oven using a validated process. Alternatively, a plastic syringe with a sterilized needle, that has been confirmed to have no detectable pyrogenic substances and no interference with the pyrogen test is available.

Procedures

1) Volume of sample injection

Unless otherwise specified, the test volume should be 10 mL of the test solution per 1 kg of the body weight of the test animal.

2) Procedure

Perform the test in a laboratory that has the same ordinary temperature as the breeding room and is free from disturbances that could agitate the test animals. Withhold all feed for the rabbits for several hours before measuring the control body temperature until the end of the test. Restrain the rabbits with light-fitting neck restraints that allow them to assume a natural resting position. Measure the body temperature by inserting a rectal thermometer or a body temperature-measuring device into the rectum at a certain depth within the range of 60 to 90 mm. When the test animal is stable, measure the body temperature and set it as the control body temperature. Then, inject the test solution within 30 minutes. The difference in control body temperature between the test animals to be injected with the test solution should be 1 ℃ or less. Animals with control body temperature higher than 39.8℃ should not be used for the test.

Warm the test solution to 37 ± 2 ℃ and slowly inject it into the ear vein of the test animal. However, note that injection for one animal should be completed within 10 minutes. Pyrogen-free sodium chloride can be added to the test solution to make it isotonic. Measure body temperature every 30 minutes up to 3 hours after injection. The difference between the control body temperature and the highest body temperature is defined as an increase in body temperature.

Interpretation of the results

The first test is carried out in a group of three rabbits. If the highest temperature is less than the control temperature, the temperature rise is considered to be 0 °C. If no animal shows a temperature increase of 0.5 ℃ or more, the test is negative for pyrogen and the test is considered to be suitable. If one or more animal shows a temperature increase of 0.5 ℃ or more, the test is repeated. The second test is carried out in a group of 5 rabbits. If the number of animals that showed a temperature increase of 0.5 °C or more is 3 or less, and if the sum of the temperature increase in the total 8 rabbits is 3.3°C or less, the test result is considered negative for pyrogen.

Qualitative Analysis
정성반응

The Qualitative Analysis is used for the identification of drugs and are usually done by taking 2 to 5 mL of the test solution specified in the monograph.

Aluminum salt 1) When ammonium chloride TS and ammonia TS are added to aluminum salt solutions, white gel-like precipitates are formed, which do not dissolve even when an excess of ammonia TS is added subsequently.

2) When sodium hydroxide TS is added to aluminum salt solutions, white gel-like precipitates are formed, which dissolve if an excess of sodium hydroxide TS is added subsequently.

3) When sodium sulfide TS is added to aluminum salt solutions, white gel-like precipitates are formed, which dissolve if an excess of sodium hydroxide TS is added subsequently.

4) Add ammonia TS to aluminum salt solutions until white gel-like precipitates are formed. Add 5 drops of alizarin S TS, and the precipitates turn red.

Ammonium salt When an excess of sodium hydroxide TS is added to ammonium salt and heated, it gives out the odor of ammonia, and this gas changes a red litmus test paper soaked in water to blue.

Antimony salt, Primary 1) Dissolve the primary antimony salt in as little hydrochloric acid as possible and dilute it with water; it becomes white and cloudy. When 1 drop of sodium nitrite TS is added to this solution, orange precipitates are formed. When the precipitates are taken separately, and sodium sulfide TS is added to a portion of the precipitates and sodium hydroxide TS is added to another portion, they dissolve in either of these reagents.

2) Add water to an acidic solution of primary antimony salt in hydrochloric acid until a small quantity of precipitates are formed, and then add sodium thiosulfate TS thereto; the precipitates dissolve. When the solution is heated, a red precipitate is formed.

Aromatic amines, Primary While cooling with ice, add 3 drops of sodium nitrite TS to the acidic solution of primary aromatic amines. Leave it to stand for 2 minutes after shaking and mixing it well. Thereto add 1 mL of ammonium sulfamate TS is added, leave it to stand again for 1 minute after shaking and mixing it well, and then add 1 mL of N-(1-naphthyl)-N'-diethyl-ethylenediamine oxalate TS. The resulting solution exhibits a reddish purple color.

Arsenate 1) When 1 to 2 drops of sodium sulfide TS are added to a neutral solution of arsenate, no precipitate is formed, but when hydrochloric acid is added subsequently, yellow precipitates are formed. When the precipitates are taken separately and ammonium carbonate TS is added to the precipitates, they dissolve.

2) When silver nitrate TS is added to a neutral solution of arsenate, dark reddish brown precipitates are formed. If dilute nitric acid is added to a portion of the precipitates and ammonia TS is added to another portion, the precipitates dissolve in either of these reagents.

3) When magnesia TS is added to a neutral arsenate solution or an alkaline solution of arsenate in ammonia, white crystalline precipitates are formed. If dilute hydrochloric acid is added subsequently, the precipitates dissolve.

Arsenite 1) When 1 to 2 drops of sodium sulfide TS are added to an acidic solution of arsenite in hydrochloric acid, yellow precipitates are formed. When the precipitates are taken separately and hydrochloric acid is added to a portion of them, they still do not dissolve. When ammonium carbonate TS is added to another portion, the precipitates dissolve.

2) When silver nitrate TS is added to a slightly alkaline solution of arsenite, yellowish white precipitates are formed. If ammonia TS is added to a portion of the solution and dilute nitric acid is added to another portion, the precipitates dissolve in either of these reagents.

3) When copper (II) sulfate TS is added to a slightly alkaline solution of arsenite, a green precipitate is formed. When the precipitates are taken separately and then boiled with sodium hydroxide TS, they turn reddish brown.

Acetate 1) When acetate is mixed with dilute sulfuric acid (1 in 2) and then heated, it gives out the odor of acetic acid.

2) When an acetate is heated with sulfuric acid and a small amount of ethanol, it gives out the odor of ethyl acetate.

3) When iron (III) chloride TS is added to a neutral solution of acetate, the solution turns reddish brown, and if this solution is boiled, reddish brown precipitates are formed. When hydrochloric acid is added to this solution, the precipitates dissolve, and the color of the solution becomes yellow.

Barium salt 1) When tested according to the Flame Coloration 1), barium salt exhibits a persistent yellowish green color.

2) When dilute sulfuric acid is added to a barium salt solution, white precipitates are formed, which do not dissolve even when dilute nitric acid is added subsequently.

3) When potassium chromate TS is added to an acidic solution of barium salt in acetic acid, yellow precipitates are formed, and if dilute nitric acid is added thereto, the precipitates dissolve.

Benzoate 1) When dilute hydrochloric acid is added to a concentrated solution of benzoate, white crystalline precipitates are formed. The precipitates are taken separately, washed thoroughly with cold water, and dried. The melting point of the resulting precipitates is between 120 °C and 124 °C.

2) When iron (III) chloride TS is added dropwise to a neutral solution of benzoate, light yellowish red precipitates are formed, and if dilute hydrochloric acid is added thereto, they turn white.

Bicarbonate 1) When dilute hydrochloric acid is added to bicarbonate, bubbles are formed and a gas is generated. When this gas is passed through calcium hydroxide TS, white precipitates are formed immediately (common with carbonates).

2) When magnesium sulfate TS is added to a bicarbonate solution, no precipitates are formed, but when it is boiled, white precipitates are formed.

3) When 1 drop of phenolphthalein TS is added to a cold solution of bicarbonate, the solution does not turn red or even if it has a red tint, it is extremely pale (distinguishable from carbonate).

Bismuth salt 1) When bismuth salt is dissolved in as little hydrochloric acid as possible and diluted with water, it becomes white and cloudy. When 1 to 2 drops of sodium sulfide TS are added to this solution, dark brown precipitates are formed.

2) When thiourea TS is added to an acidic solution of bismuth salt in hydrochloric acid, the solution turns yellow.

3) When potassium iodide TS is added dropwise to dilute nitric acid solution or dilute sulfuric acid solution of bismuth salt, black precipitates are formed. If potassium iodide TS is added subsequently, the precipitates dissolve, and the solution turns orange.

Borate 1) When borate is mixed with sulfuric acid and methanol, and then ignited, it shows a green flame.

2) When a turmeric test paper soaked in an acidic solution of borate in hydrochloric acid is heated and dried, it turns red, and if ammonia TS is added thereto dropwise, it turns blue.

Bromate 1) When 2 to 3 drops of silver nitrate solution are

added to an acidic solution of bromate in nitric acid, white crystalline precipitates are formed, which dissolve when heated. When 1 drop of sodium nitrite TS is added to this solution, pale yellow precipitates are formed.

2) When 5 to 6 drops of sodium nitrite TS are added to an acidic solution of bromate in nitric acid, the solution turns yellow to reddish brown, and if 1 mL of chloroform is added thereto and the solution is mixed and shaken, the chloroform layer turns yellow to reddish brown.

Bromide 1) When silver nitrate solution is added to a bromide, pale yellow precipitates are formed. When the precipitates are taken separately and dilute nitric acid is added to a portion of the precipitates, they still do not dissolve. When ammonia water (28) is added to another portion, which is then mixed thoroughly by shaking, and the solution isolated from the mixture is acidified by adding dilute nitric acid, the resulting solution becomes white and cloudy.

2) When chlorine TS is added to a bromide solution, it turns yellowish brown. When chloroform is added to a portion of this solution, which is then mixed and shaken, the chloroform layer shows a yellowish brown to reddish brown color. When phenol is added to another portion, white precipitates are formed.

Calcium salt 1) When tested according to the Flame Coloration 1), calcium salt exhibits a yellowish red color.

2) When ammonium carbonate TS is added to a calcium salt solution, white precipitates are formed.

3) When ammonium oxalate TS is added to a calcium salt solution, white precipitates are formed. When the precipitates are taken separately and dilute acetic acid is added to them, they do not dissolve, but they do if dilute hydrochloric acid is added.

4) When a neutral solution of calcium salt is mixed with 10 drops of potassium chromate TS and then heated, no precipitate is formed (distinguishable from strontium salt).

Carbonate salt 1) When dilute hydrochloric acid is added to carbonate salt, bubbles are formed and gas is generated. When this gas is passed through calcium hydroxide TS, white precipitates are formed immediately (common with bicarbonates).

2) When magnesium sulfate TS is added to a carbonate solution, white precipitates are formed, which dissolve if dilute acetic acid is added subsequently.

3) When 1 drop of phenolphthalein TS is added to a cold solution of carbonate, the solution turns red (distinguishable from bicarbonate).

Cerous salt 1) When 2.5 times the amount of lead (IV) oxide is added to cerous salt, thereto nitric acid is added, and then the mixture is boiled, the solution turns yellow.

2) When hydrogen peroxide TS and ammonia TS are added to a cerous salt solution, yellow to reddish brown precipitates are formed.

Chlorate 1) When silver nitrate TS is added to a chlorate solution, no precipitate is formed, but when 2 to 3 drops of sodium nitrite TS and dilute nitric acid are added subsequently, white precipitates are slowly formed. If ammonia TS is added thereto, the precipitates dissolve.

2) Add indigo carmine TS dropwise to a neutral solution of chlorate until a pale blue color appears. Add dilute sulfuric acid to make it acidic, and then add sodium bisulfite TS dropwise; the blue color disappears promptly.

Chloride 1) When a chloride solution is mixed with sulfuric acid and potassium permanganate and heated, chlorine gas is generated, and this gas turns the potassium iodide starch paper soaked in water blue.

2) When silver nitrate TS is added to a chloride solution, white precipitates are formed. When the precipitates are taken separately and dilute nitric acid is added to a portion of the precipitates, they still do not dissolve. When an excess of ammonia TS is added to another portion, the precipitates dissolve.

Chromate 1) A chromate solution exhibits a yellow color.

2) When lead acetate TS is added to a chromate solution, yellow precipitates are formed, and when acetic acid is added to a portion of this solution, the precipitates still do not dissolve. When dilute nitric acid is added to another portion, the precipitates dissolve.

3) When an equal volume of ethyl acetate and 1 to 2 drops of peroxide TS are added to an acidic solution of chromate in sulfuric acid, and the resulting solution is mixed and shaken immediately and left to stand, the ethyl acetate layer exhibits a blue color.

Citrate 1) When 20 mL of a mixture of pyridine and acetic anhydride (3:1) is added to 1 to 2 drops of citrate solution, and then left to stand for 2-3 minutes, it turns reddish brown.

2) When a neutral solution of citrate is mixed with the same amount of dilute sulfuric acid, to which 2/3 the volume of potassium permanganate TS is added, then heated until the color of the test solution disappears, and thereto 1/10 the volume of bromine TS is added dropwise, white precipitates are formed.

3) When a neutral solution of citrate is mixed with an excess of calcium chloride TS and boiled, white crystalline precipitates are formed. When the precipitates are taken separately and sodium hydroxide TS is added to a portion of them, they still do not dissolve. When dilute hydrochloric acid is added to another portion, the precipitate dissolves.

Cupric salt 1) When a well-polished plate-shaped piece of iron is placed into an acidic solution of cupric salt in hydrochloric acid, a red metallic film is formed on its surface.

2) When a small amount of ammonia TS is added to a cupric salt solution, pale blue precipitates are formed, and when an excess of ammonia TS is added subsequently, the precipitates dissolve, and the solution turns dark blue.

3) When potassium potassium hexacyanoferrate(II) TS is added to a cupric salt solution, reddish brown precipitates are formed, and even when dilute nitric acid is added to a portion of this solution, the precipitates still do not dissolve. When ammonia TS is added to another portion, the precipitates dissolve, and the solution turns dark blue.

4) When sodium sulfide TS is added to a cupric salt solution, black precipitates are formed. When the precipitates are taken separately and dilute hydrochloric acid, dilute sulfuric acid, or sodium hydroxide TS is added to a portion of the precipitates, they still do not dissolve. When hot dilute nitric acid is added to another portion of the precipitates, the precipitates dissolve.

Cyanide 1) When an excess of silver nitrate TS is added to a cyanide solution, white precipitates are formed. When the precipitates are taken separately and dilute nitric acid is added to a portion of them, they still do not dissolve. When ammonia TS is added to another portion, the precipitates dissolve.

2) When 2 to 3 drops of iron (II) sulfate TS, 2 to 3 drops of dilute iron (III) chloride TS, and 1 mL of sodium hydroxide TS are added to a cyanide solution, which is then mixed by shaking and acidified by adding dilute sulfuric acid; blue precipitates are

formed.

Dichromate 1) A dichromate solution exhibits a yellowish red color.

2) When lead acetate TS is added to a dichromate solution, yellow precipitates are formed, and when acetic acid is added to a portion of this solution, the precipitates still do not dissolve. When dilute nitric acid is added to another portion, the precipitates dissolve.

3) When an equal volume of ethyl acetate and 1 to 2 drops of peroxide TS are added to an acidic solution of dichromate in sulfuric acid, and the resulting solution is mixed and shaken immediately and left to stand, the ethyl acetate layer exhibits a blue color.

Ferric salt 1) When potassium potassium hexacyanoferrate(II) TS is added to a slightly acidic solution of ferric salt, blue precipitates are formed, which do not dissolve even when dilute hydrochloric acid is added.

2) When sodium hydroxide TS is added to a ferric salt solution, reddish brown gel-like precipitates are formed, and when sodium sulfide TS is added, they turn black. When the precipitates are taken separately and dilute hydrochloric acid is added to them, they dissolve, and the solution becomes white and cloudy.

3) When sulfosalicylic acid solution is added to a slightly acidic solution of ferric salt, the solution turns purple.

Ferricyanide 1) A ferricyanide solution exhibits a yellow color.

2) When iron (II) sulfate TS is added to a ferricyanide solution, blue precipitates are formed, which do not dissolve even when dilute hydrochloric acid is added subsequently.

Ferrocyanide 1) When iron (III) chloride TS is added to a ferrocyanide solution, blue precipitates are formed, which do not dissolve even when dilute hydrochloric acid is added subsequently.

2) When copper (II) sulfate TS is added to a ferrocyanide solution, reddish brown precipitates are formed, which do not dissolve even when dilute hydrochloric acid is added subsequently.

Ferrous salt 1) When potassium hexacyanoferrate(III) TS is added to a slightly acidic solution of ferrous salt, blue precipitates are formed, which do not dissolve even when dilute hydrochloric acid is added.

2) When sodium hydroxide TS is added to a ferrous salt solution, gray-green gel-like precipitates are formed, which turn black if sodium sulfide TS is added thereto. When the precipitates are taken separately and dilute hydrochloric acid is added to them, they dissolve.

3) When an ethanol solution (1 in 50) of *o*-phenanthroline is added dropwise to a neutral or slightly acidic solution of ferrous salt, it turns dark red.

Fluoride 1) When a fluoride solution is mixed with chromic acid-sulfuric acid TS and then heated, the liquid does not coat the inner wall of the test tube evenly.

2) When 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer (pH 4.3), and cerium (III) nitrate TS (1:1:1) is added to a neutral or slightly acidic solution of fluoride, the solution turns bluish purple.

Glycerophosphate 1) When calcium chloride TS is added to a glycerophosphate solution, no change is observed, but when it is boiled, precipitates are formed.

2) When ammonium molybdate TS is added to a cold glycerophosphate solution, no precipitates are formed. However, yellow precipitates are formed when it is boiled for a long time.

3) When glycerophosphate is mixed with an equal amount of potassium hydrogen sulfate powder and heated gently over direct heat, it gives out the pungent odor of acrolein.

Iodide 1) When silver nitrate TS is added to an iodide solution, yellow precipitates are formed. When dilute nitric acid is added to a portion of this solution, and ammonia water (28) is added to another portion, the precipitates dissolve in neither of these reagents.

2) When 1 to 2 drops of sodium nitrite TS are added to an acidic solution of iodide, the solution turns yellow -brown, and then black-purple precipitates are formed. When starch TS is added subsequently, the solution turns dark blue.

Lactate When an acidic solution of lactate in sulfuric acid is mixed with potassium permanganate TS and heated, it gives out the odor of acetaldehyde.

Lead salt 1) When dilute sulfuric acid is added to a lead salt solution, white precipitates are formed. When the precipitates are taken separately and dilute nitric acid is added to a portion of the precipitates, they still do not dissolve. If sodium hydroxide TS is added to another portion of the precipitates, and then heated or ammonium acetate TS is added to the mixture, the precipitates dissolve.

2) When sodium hydroxide TS is added to a lead salt solution, white precipitates are formed, and if an excess of sodium hydroxide TS is added thereto, the precipitates dissolve. Then, if sodium sulfide TS is added thereto, black precipitates are formed.

3) When potassium chromate TS is added to an acidic solution of lead salt in a dilute acetic acid solution, yellow precipitates are formed. These precipitates do not dissolve even when ammonia TS is added, but they dissolve when sodium hydroxide TS is added thereto subsequently.

Lithium salt 1) When tested according to the Flame Coloration 1) lithium salt exhibits a persistent red color.

2) When disodium hydrogen phosphate TS is added to a lithium salt solution, white precipitates are formed, and if dilute hydrochloric acid is added thereto, the precipitates dissolve.

3) Precipitation does not occur when dilute sulfuric acid is added to a lithium salt solution (distinguishable from strontium salt).

Magnesium salt 1) When a magnesium salt solution is mixed with ammonium carbonate TS and heated, white precipitates are formed, and if ammonium chloride TS is added thereto subsequently, the precipitates dissolve. If disodium hydrogen phosphate TS is added thereto, white crystalline precipitates are formed.

2) When sodium hydroxide TS is added to a magnesium salt solution, white gel-like precipitates are formed, and if iodine TS is added to a portion of the precipitates, they develop a dark-brown color. When an excess of sodium hydroxide TS is added to another portion of the precipitates, they still do not dissolve.

Manganese salt 1) When ammonia TS is added to a manganese salt solution, white precipitates are formed. When silver nitrate TS is added to a portion of the solution, the precipitates turn black. When another portion is left to stand, the upper part of the precipitates turns brown.

2) When a small amount of sodium bismuthate powder is added to an acidic solution of manganese salt in dilute nitric acid, the solution turns reddish purple.

Mercuric salt 1) When a copper plate is immersed in a mercuric salt solution, left to stand for a while, then washed with water, and wiped with paper or cloth, the plate becomes bright and silvery white in appearance (common with mercurous salt).

2) When a small amount of sodium sulfide TS is added to a mercuric salt solution, black precipitates are formed, and they dissolve when an excess of sodium sulfide TS is added subsequently. When ammonium chloride TS is added to this solution, black precipitates are formed again.

3) When potassium iodide TS is added dropwise to a neutral solution of mercuric salt, red precipitates are formed, and if an excess of potassium iodide TS is added subsequently, the precipitates dissolve.

4) When a small amount of stannous chloride TS is added to an acidic solution of mercuric salt in hydrochloric acid, white precipitates are formed, and if an excess of stannous chloride TS is added subsequently, the precipitates turn blackish gray.

Mercurous salt 1) When a copper plate is immersed in a mercurous salt solution, left to stand for a while, then washed with water, and wiped with paper or cloth, the plate becomes bright and silvery white in appearance (common with mercuric salt).

2) When sodium hydroxide TS is added to mercurous salt or its solution, it turns black.

3) When dilute hydrochloric acid is added to a mercurous salt solution, white precipitates are formed. When the precipitates are taken separately and ammonia TS is added to them, they turn black.

4) When potassium iodide TS are added to a mercurous salt solution, yellow precipitates are formed. When left to stand, the precipitates turn green, and if an excess of potassium iodide TS is added subsequently, it turns black.

Mesylate 1) Add twice the amount of sodium hydroxide to the mesylate, gradually heat to dissolve, and continue heating for 20 to 30 seconds. After cooling, add a small amount of water and dilute hydrochloric acid to the mixture to warm it. A gas is generated, which makes moistened potassium iodate starch paper blue.

2) Add 3 times the amount of sodium nitrate and 3 times the amount of anhydrous sodium carbonate to the mesylate, mix them thoroughly by shaking, and heat the mixture slowly. After cooling, dissolve the residue in dilute hydrochloric acid (1 in 5. The resulting solution is filtered, if necessary, and then barium chloride TS is added to the filtrate, and white precipitates are formed.

Nitrate 1) When a nitrate solution is mixed with an equal volume of sulfuric acid, cooled, and then layered with, a dark-brown band is formed at the interface of the solution.

2) When diphenylamine TS is added to a nitrate solution, it turns blue.

3) When potassium permanganate TS is added to an acid solution of nitrate in sulfuric acid, the reddish purple color of the test solution does not fade (distinguishable from nitrite).

Nitrite 1) When a nitrite solution is acidified by adding dilute sulfuric acid, a yellowish brown gas with a peculiar odor is generated. If a small amount of crystals of iron (II) sulfate heptahydrate are added thereto, the solution turns dark brown.

2) When 2 to 3 drops of potassium iodide TS are added to a nitrite solution and thereto dilute sulfuric acid is added dropwise, the solution turns yellowish brown, and then black-purple precipitates are formed. If 2 mL of chloroform is added thereto and mixed by shaking, the chloroform layer exhibits a purple color.

3) Add thiourea TS to a nitrite solution, add dilute sulfuric acid to acidify it, and thereto add iron (III) chloride TS dropwise; the solution turns dark red. Then, if 2 mL of ether is added thereto and mixed and shaken; the ether layer exhibits a red color.

Oxalate 1) When potassium permanganate TS is added dropwise to a warm acidic solution of oxalate in sulfuric acid, the color of the test solution disappears.

2) When calcium chloride TS is added to oxalate solutions, white precipitates are formed. When the precipitates are taken separately and dilute acetic acid is added to the precipitates, they do not dissolve, but they dissolve if dilute hydrochloric acid is added.

Permanganate 1) A permanganate solution exhibits a reddish purple color.

2) When an acidic solution of permanganate in sulfuric acid is mixed with an excess amount of hydrogen peroxide TS, the solution effervesces and loses its color.

3) When an acidic solution of permanganate in sulfuric acid is mixed with an excess amount of oxalic acid TS and then heated, the solution loses its color.

Peroxide 1) Add 1 to 2 drops of ethyl acetate and potassium dichromate solution in the same amount to the peroxide solution, add dilute sulfuric acid to make it acidic, shake immediately, mix, and leave to stand., the ethyl acetate layer exhibits a blue color.

2) When potassium permanganate TS is added dropwise to an acidic solution of peroxide in sulfuric acid, the solution loses its color and generates a gas with bubbles.

Phosphate (Orthophosphate) 1) When silver nitrate TS is added to a neutral phosphate solution, yellow precipitates are formed. If dilute nitric acid or ammonia TS is added subsequently, the precipitates dissolve.

2) When a neutral phosphate solution or an acidic phosphate solution in dilute nitric acid is mixed with ammonium molybdate TS and heated, yellow precipitates are formed. If sodium hydroxide TS or ammonia TS is added thereto, the precipitates dissolve.

3) When magnesia TS is added to a neutral phosphate solution or an alkaline ammonia phosphate solution, white crystalline precipitates are formed. If dilute hydrochloric acid is added subsequently, the precipitates dissolve.

Potassium salt 1) When tested according to the Flame Coloration 1), potassium salt exhibits a pale purple color. When the flame is yellow, a reddish purple color can be seen through the cobalt glass.

2) When sodium hydrogen tartrate TS is added to a neutral solution of potassium salt, white crystalline precipitates are formed. The formation is accelerated by rubbing the inside wall of the test tube with a glass rod. When the precipitates are taken separately, and ammonia TS, sodium hydroxide TS, or sodium carbonate TS is added to them, they dissolve in any of these reagents.

3) When sodium cobalt nitrite TS is added to an acidic solution of potassium salt in acetic acid, yellow precipitates are formed.

4) When potassium salt is mixed with an excess of sodium

hydroxide TS and heated, it has no ammonia odor (distinguishable from ammonium salt).

Salicylate 1) When salicylate is mixed with an excess of soda lime and heated, a phenol-like odor comes out.

2) When dilute hydrochloric acid is added to a concentrated solution of salicylate, white crystalline precipitates are formed. When the precipitates are taken separately, then washed thoroughly with cold water, and dried, the melting point of the resulting precipitates is around 159 °C.

3) When 5 to 6 drops of dilute iron (III) chloride TS are added to a neutral solution of salicylate, the solution turns red. As dilute hydrochloric acid is added thereto drop by drop, the color of the solution changes to purple first and then disappears.

Silver salt 1) When dilute hydrochloric acid is added to a silver salt solution, white precipitates are formed, and the precipitates do not dissolve even when dilute nitric acid is added to a portion of it. When an excess of ammonia TS is added to another portion, the precipitates dissolve.

2) When potassium chromate TS is added to a silver salt solution, red precipitates are formed, and if dilute nitric acid is added thereto, the precipitates dissolve.

3) When ammonia TS is added drop by drop to a silver salt solution, gray-brown precipitates are formed. Then, ammonia TS is added dropwise thereto until the precipitates dissolve. When 1 to 2 drops of formaldehyde solution are added and heated, a mirror of metallic silver is formed on the inner wall of the container.

Sodium salt 1) When tested according to the Flame Coloration 1), sodium salt exhibits a yellow color.

2) When potassium hexahydroxoantimonate (V) TS is added to a neutral or slightly alkaline concentrated solution of sodium salt, white crystalline precipitates are formed. The formation of the precipitates is accelerated by rubbing the inner wall of the test tube with a glass rod.

Stannic salt 1) When an acidic solution of stannic salt in hydrochloric acid is coated on the outer bottom of a test tube filled with water, and then placed into a non-luminous flame of a Bunsen burner, a blue flame mantle surrounds the bottom of the test tube (common with stannous salt).

2) When granular zinc is immersed in an acidic solution of stannic salt in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannous salts).

3) Add iron powder to an acidic solution of stannic salt in hydrochloric acid, leave it to stand, and then filter it. When iodine-starch TS is added dropwise to the filtrate, the color of the test solution disappears.

4) Add ammonia TS drop by drop to an acidic solution of stannic salt in hydrochloric acid until a small quantity of precipitates is produced, and thereto add 2 to 3 drops of sodium sulfide TS. Pale yellow precipitates are formed. When the precipitates are taken separately and dilute sodium sulfide TS is added to the precipitates, they dissolve. If hydrochloric acid is added subsequently, pale yellow precipitates are formed again.

Stannous salt 1) When an acidic solution of stannous salt in hydrochloric acid is coated on the outer bottom of a test tube filled with water, and then placed into a non-luminous flame of a Bunsen burner, a blue flame mantle surrounds the bottom of the test tube (common with stannic salt).

2) When granular zinc is immersed in an acidic solution of stannous salt in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannic salts).

3) When iodine-starch TS is added dropwise to a stannous salt solution, the color of the test solution disappears.

4) Add ammonia TS dropwise to an acidic solution of stannous salt in hydrochloric acid until a small quantity of precipitates is produced, and thereto add 2 to 3 drops of sodium sulfide TS. Dark brown precipitates are formed. When the precipitates are taken separately and dilute sodium sulfide TS is added to a portion of the precipitates, they still do not dissolve. When ammonium polysulfide TS is added to another portion, the precipitates dissolve.

Sulfate 1) When barium chloride TS is added to a sulfate solution, white precipitates are formed, which do not dissolve even when dilute nitric acid is added subsequently.

2) When lead acetate TS is added to a neutral solution of sulfate, white precipitates are formed, which dissolve if ammonium acetate TS is added.

3) When an equal volume of dilute hydrochloric acid is added to a sulfate solution, the solution does not become white and cloudy (distinguishable from thiosulfate). Also, it does not smell like sulfur dioxide (distinguishable from sulfite).

Sulfide When dilute hydrochloric acid is added, many sulfides smell like hydrogen sulfide, and such gases turn lead acetate test paper soaked in water black.

Sulfite and bisulfite 1) When iodine TS is added drop by drop to an acidic solution of sulfite or bisulfite in acetic acid, the color of the test solution disappears.

2) When an equal volume of dilute hydrochloric acid is added to a sulfite or bisulfite solution, it gives out the odor of sulfur dioxide and the solution is not turbid (distinguishable from thiosulfates). When 1 drop of sodium sulfide TS is added, the solution soon becomes white and cloudy, which slowly turn into pale yellow precipitates.

Tartrate 1) When silver nitrate TS is added to a neutral solution of tartrate, white precipitates are formed. When the precipitates are taken separately and nitric acid is added to a portion of them, they dissolve. When ammonia TS is added to another portion and the mixture is heated, the precipitates dissolve, and silver speckles are formed slowly on the inner wall of the test tube, forming a mirror.

2) When 2 drops of acetic acid (31), 1 drop of iron (II) sulfate TS, and 2 to 3 drops of hydrogen peroxide TS are added to a tartrate solution, to which an excess of sodium hydroxide TS is added, the solution turns reddish purple to purple.

3) When 2 to 3 drops of a tartrate solution are mixed with a solution prepared by mixing 2 to 3 drops of resorcinol solution (1 in 50) and 2 to 3 drops of potassium bromide solution (1 in 10) with 5 mL of sulfuric acid and then heated for 5 to 10 minutes on a water bath, the solution exhibits a dark blue color. After this solution is cooled down, 3 mL of water is added to it, and the resulting solution turns red to reddish orange.

Thiocyanate 1) When an excess of silver nitrate TS is added to a thiocyanate solution, white precipitates are formed. When dilute nitric acid is added to a portion of this solution, they still do not dissolve. When ammonia water (28) is added to another portion, the precipitates dissolve.

2) When iron (III) chloride TS is added to a thiocyanate solution, a red color appears, and this color does not disappear even when hydrochloric acid is added.

Thiosulfate 1) When iodine TS is added drop by drop to an acidic solution of Thiosulfate in acetic acid, the color of the test solution disappears.

2) When an equal volume of dilute hydrochloric acid is added to a thiosulfate solution, it gives out the odor of sulfur dioxide, and the solution gradually becomes turbid and white, which later turns yellow if it is left to stand.

3) When an excess of silver nitrate TS is added to a thiosulfate solution, white precipitates are formed, which turn black when left to stand.

Zinc salt 1) When ammonium sulfide TS or sodium sulfide TS is added to a neutral to alkaline solution of zinc salt, white precipitates are formed. When the precipitates are taken separately and dilute acetic acid is added to them, they do not dissolve, but they dissolve if dilute hydrochloric acid is added thereto.

2) When potassium potassium hexacyanoferrate(II) TS is added to a zinc salt solution, white precipitates are formed. When dilute hydrochloric acid is added to a portion of the solution, the precipitates still do not dissolve. When sodium hydroxide TS is added to another portion, the precipitates dissolve.

3) When 1 to 2 drops of pyridine and 1 mL of potassium thiocyanate TS are added to a neutral to slightly acidic solution of zinc salt, white precipitates are formed.

Readily Carbonizable Substances
황산에 의한 정색물시험법

The Readily Carbonizable Substances is a method to examine the minute impurities contained in drugs, which are readily colored when adding sulfuric acid.

Procedure

Before use, wash the Nessler tubes thoroughly with sulfuric acid for readily carbonizable substances. Unless otherwise specified, proceed as follows. When the sample is solid, add 5 mL of sulfuric acid for readily carbonizable substances to a Nessler tube, add the specified quantity of the finely powdered sample, little by little, as directed in the monograph, and dissolve it completely by stirring with a glass rod. When the sample is liquid, take the amount of the sample specified in the monograph, to a Nessler tube, add 5 mL of sulfuric acid for readily carbonizable substances, and mix by shaking. If the temperature of the content rises, cool the content; and if the reaction is affected by temperature, maintain it at the standard temperature. Allow to stand for 15 minutes, and compare the color of the liquid with the color of the control solution in the Nessler tube as specified in the monograph, by viewing transversely against a white background.

Refractive Index
굴절률측정법

The Refractive Index is to measure the refractive index of the sample with respect to air. Generally, when a ray of light travels from one medium to another, its direction of travel is generally changed at the boundary surface. This phenomenon is called refraction.

When a ray of light enters the second medium from an isotropic first medium, the ratio of the sine value of the angle of incidence, *i*, to that of the angle of refraction, *r*, is constant between the two media, regardless of the angle of incidence. This ratio is referred to as the refractive index of the second medium with respect to the first, or the relative refractive index, and is denoted by the symbol *n*.

$$n = \frac{\sin i}{\sin r}$$

The refractive index when the first medium is vacuum is referred to as the absolute refractive index of the second medium, denoted by the symbol *N*.

For isotropic substances, when wavelength, temperature, and pressure are constant, the refractive index is a unique constant to the substance. Therefore, this measurement is applied to test the purity of substances or to determine the composition of homogeneous mixtures consisting of two substances.

The temperature used is usually 20 ℃, and the D line of the sodium spectrum is utilized for irradiation. This value is denoted by the symbol n_D^{20}.

Procedure

The refractive index is usually measured using the Abbe refractometer within a range of ± 0.2 ℃ of the temperature specified in the monograph. The Abbe refractometer uses white light to read n_D directly, and can measure a range of 1.3 to 1.7with a precision of 0.0002.

Residual Solvents
잔류용매시험법

For pharmacopeial purposes, residual solvents in pharmaceuticals are defined as volatile organic chemicals that are used or produced in the manufacturing of drug substances, or excipients, or in the preparation of drug products, and are measured by gas chromatography.

Unless otherwise specified, the limit of residual – solvents in pharmaceuticals is expressed in ppm and is NMT the concentration limit in the 「Guideline for Residual Solvents in Pharmaceuticals」.

1. Solvents of Class 1 and Class 2

Apparatus

Gas Chromatography

Procedure

1) Preparation of standard solutions

a) Class 1 standard stock solutions:

Dissolve benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethane, and 1,1,1-trichloroethane in dimethyl sulfoxide to make 10,000 ppm, 20,000 ppm, 25,000 ppm, 40,000 ppm, and 50,000 ppm, respectively. Take exactly 1 mL of this solution, add exactly 9 mL of dimethyl sulfoxide, and add water to make it up to 100 mL. Take exactly 1 mL of this solution and add water to make it up to 100 mL. After taking exactly 1 mL of this solution, add water to make 10 mL and use it as Class 1 standard stock solution. However, when testing a sample that is insoluble in water, use dimethylformamide or dimethyl sulfoxide instead of water to prepare the standard stock solution.

b) Class 1 standard solution

Transfer 1 mL of Class 1 standard stock solution to a headspace vial containing 5 mL of water. Apply the stopper, cap, and mix, and use it as the Class 1 standard solution.

c) Class 2 standard stock solution

Dissolve acetonitrile, chlorobenzene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,4-dioxane, methanol, methylcyclohexane, toluene, xylene, and tetrahydrofuran in dimethyl sulfoxide to make 2,050 ppm, 1,800 ppm, 19,400 ppm, 9,350 ppm, 3,000 ppm, 1,900 ppm, 15,000 ppm, 5,900 ppm, 4,450 ppm, 10,850 ppm, and 3,600 ppm, respectively. Take exactly 1 mL of this solution, and add water to make it up to 100 mL and use it as the Class 2 standard stock solution (I). Separately, dissolve chloroform, 1,2-dimethoxyethane, hexane, methylbutyl ketone, nitromethane, pyridine, tetralin, 1,1,2-trichloroethene in dimethyl sulfoxide to make 300 ppm, 500 ppm, 1,450 ppm, 250 ppm, 250 ppm, 1,000 ppm, 500 ppm, and 400 ppm, respectively. Take exactly 1 mL of this solution, add water to make it up to 100 mL, and use it as the Class 2 standard stock solution (II). However, when testing a sample that is insoluble in water, use dimethylformamide or dimethyl sulfoxide instead of water to prepare the standard stock solution.

d) Class 2 standard solution

Transfer 1 mL of Class 2 standard stock solution (I) to a headspace vial containing 5 mL of water, apply the stopper, cap, and mix. Use it as Class 2 standard solution (I). Separately, transfer 5 mL of Class 2 standard stock solution (II) to a headspace vial containing 1 mL of water. Apply the stopper, cap, and mix, and use it as the Class 2 standard solution (II).

e) Standard solution by component

For the components detected following the Stage 1 and Stage 2 tests, dissolve each in dimethyl sulfoxide by referencing the concentration of the first dilution step of the standard stock solution of Class 1 or Class 2. Take 1 mL of this solution, and dilute it with water at each stage to a concentration similar to the concentration detected in the test and standard solution during the Stage 1 and Stage 2 tests. If necessary, dilute it up to 20 times the concentration limit specified in the "Guidelines for Residual Solvents in Pharmaceuticals." However, when testing a sample that is insoluble in water, use dimethylformamide or dimethyl sulfoxide instead of water to dissolve. Transfer 1 mL of this solution to a headspace vial containing 5 mL of water. Apply the stopper, cap, and mix, and use it as the standard solution for each component.

2) Preparation of test solution

a) Water-soluble sample

① Sample stock solution

Take the sample as it is or more than a certain amount, if necessary, make it into powder and then accurately weigh about 250 mg of it and add enough water to make it up to 25 mL while being careful not to lose the solvent. Use this as the sample stock solution.

② Test solution

Transfer 5 mL of the sample stock solution to a headspace vial containing 1 mL of water. Apply the stopper, cap, and mix, and use it as the test solution.

③ Test solution containing the standard solution

Transfer 5 mL of the sample stock solution and 1 mL of the standard solution for each component to a headspace vial. Apply the stopper, cap, and mix, and use it as the test solution containing the standard solution.

b) Water insoluble sample

① Sample stock solution

Take the sample as it is or at a certain amount or more, if necessary, make it into powder and then accurately weigh about 500 mg of it and add enough dimethylformamide or dimethyl sulfoxide to make 10 mL while being careful not to lose the solvent. Use this as the sample stock solution.

② Test solution

Transfer 1 mL of sample stock solution to a headspace vial containing 5 mL of water. Apply the stopper, cap, and mix, and use it as the test solution.

③ Test solution containing the standard solution

Transfer 1 mL of the sample stock solution, and 1 mL of standard solution for each component to a headspace vial containing 4 mL of water. Apply the stopper, cap, and mix, and use it as the test solution containing the standard solution.

3) Gas chromatography operating conditions

Among the operating conditions, the internal diameter and length of the column, the particle size of the packing material, the concentration of the stationary phase, the column temperature and the flow rate of carrier gas may be modified within limits which allow specified order of elution, resolution, the symmetry factor, and relative standard deviation to be obtained. In addition, the split ratio may be adjusted to obtain optimum sensitivity. The sample injection port and the operating conditions for headspace may also be modified within limits that allow for greater accuracy and precision more than those of a prescribed method to be achieved.

a) Method 1

Detector: Flame ionization detector.

Column: 0.32 mm x 30 m fused silica column with a 1.8 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography. Or a 0.53 mm x 30 m wide-bore column with a 3.0 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography.

Column temperature: Maintain the temperature at 40 °C for 20 minutes, then raise it to 240 °C at a rate of 10 °C per minute, and maintain it at 240 °C for 20 minutes.

Sample injection port temperature: 140 °C

Detector temperature: 250℃

Carrier gas: Helium or nitrogen

Split ratio: About 1:5

Conditions of sample injection apparatus for headspace: One of the following conditions shall be followed; however, Condition 3 must be followed when testing water-insoluble samples.

	Condition 1	Condition 2	Condition 3
Equilibrium temperature (℃)	80	105	80
Equilibrium time (min)	60	45	45
Sample injection port and connector temperature (℃)	85	110	105
Headspace vial injector port temperature (℃)	80-90	105-115	80-90
Minimum pressurization time (seconds)	60	60	60
Injection volume (mL)	1	1	1

System suitability

Test for required detectability: The signal-to-noise ratio of the 1,1,1-trichloroethane peak in the Class 1 standard solution is 5 or more, and the signal-to-noise ratio of each peak in the Class 1 system suitability solution is 3 or more.

System performance: The resolution between acetonitrile and cis-dichloromethane in the Class 2 standard solution (I) is 1.0 or higher.

• *System suitability solution:* Transfer 1 mL of the Class 1 standard stock solution to an appropriate headspace vial, add 5 mL of the test and standard solution, apply the stopper, cap, and mix. However, when testing a water insoluble sample, mix 0.5 mL of the solution just before the final dilution step of the Class 1 standard stock solution, and then 5 mL of the sample stock solution. Transfer 1 mL of this solution to an appropriate headspace vial, add 5 mL of water, and apply the stopper, cap, and mix.

b) Method 2

Detector: Flame Ionization detector.

Column: 0.32 mm x 30 m fused silica column with a 2.5 μm internal layer composed of polyethylene glycol compounds (average molecular weight is about 15,000) for gas chromatography. Or, a 0.53 mm x 30 m wide-bore column with a 2.5 μm internal layer composed of polyethylene glycol compounds (average molecular weight is about 15,000) for gas chromatography.

Column temperature: Maintain the temperature at 50 °C for 20 minutes then, raise it to 165 °C at a rate of 6 °C per minute, and maintain it at 165 °C for 20 minutes.

Sample injection port temperature: 140 °C

Detector temperature: 250 °C

Carrier gas: Helium or nitrogen

Split ratio: About 1:5

Conditions of sample injection apparatus for headspace: One of the following conditions shall be followed; however, Condition 3 must be followed when testing water-insoluble samples.

	Condition 1	Condition 2	Condition 3
Equilibrium temperature (°C)	80	105	80
Equilibrium time (min)	60	45	45
Sample injection port and connector temperature (°C)	85	110	105
Headspace vial injector port temperature (°C)	80-90	105-115	80-90
Minimum pressurization time (seconds)	60	60	60
Injection volume (mL)	1	1	1

System suitability

Test for required detectability: The signal-to-noise ratio of the benzene peak in the Class 1 standard solution is 5 or more, and the signal-to-noise ratio of each peak in the system suitability solution is 3 or more.

System performance: The resolution of acetonitrile and cis-dichloromethane in the Class 2 standard solution (I) is NLT 1.

System suitability solution: Transfer 1 mL of the Class 1 standard stock solution to an appropriate headspace vial, add 5 mL of the test and standard solution, apply the stopper, cap, and mix. However, when testing a water insoluble sample, mix 0.5 mL of the solution just before the final dilution step of the Class 1 standard stock solution and 5 mL of the sample stock solution. Transfer 1 mL of this solution to an appropriate headspace vial, add 5 mL of water, and apply the stopper, cap, and mix.

4) Procedures

Stage 1 Test

The test solution, Class 1 standard solution, Class 2 standard solution (I), and Class 2 solvent standard solution (II) are tested using the gas chromatography method, following the gas chromatography operating conditions described in Method 1. The peak area is then calculated from each chromatogram. In the test and standard solution, the peak area of each component except for 1,1,1-trichloroethane is smaller than the peak area of each component in the Class 1 standard solution and Class 2 standard solution (I) and (II). Furthermore, the peak area of 1,1,1-trichloroethane is less than 150 times the peak area of 1,1,1-trichloroethane in the Class 1 standard solution.

Stage 2 Test

If, during the Stage 1 test, the peak area of each component in the test and standard solution, except for 1,1,1-trichloroethane, is equivalent to or larger than the peak area of each component in the Class 1 standard solution and Class 2 standard solution (I) and (II), or if the peak area of 1,1,1-trichloroethane is equivalent to or larger than 150 times the peak area of 1,1,1-trichloroethane in the Class 1 standard solution during the Stage 1 test, it is retested under the gas chromatography operating conditions described in Method 2. For the components identified when tested according to Method 1, the peak area of the component in the test and standard solution is smaller than the peak area of the corresponding component in the standard solution when tested according to Method 2.

Stage 3 Test

If, during the Stage 2 test, the peak area of the component in the test solution is equal to or greater than the peak area of the corresponding component in the standard solution, the detected components are tested following the gas chromatography operating conditions described in Method 1 using the test solution, the standard solution for each component, and the test solution containing the standard solution. The amount of residual solvent in the test solution is calculated from the peak areas A_T and A_{ST} in the test solution and the test solution containing the standard solution.

2. Solvents of Class 2 and Class 3

Apparatus

Gas Chromatography

Procedure

1) Preparation of standard solutions

a) Preparation of Class 2 and Class 3A standard solutions

① Class 2A standard stock solution

Dissolve acetonitrile, chlorobenzene, cumene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,4-dioxane, methanol, methylcyclohexane, tetrahydrofuran, toluene, and xylene in dimethyl sulfoxide. Each solution is diluted as shown in the table below, and then used as the Class 2A standard stock solution.

Solvent	Concentration of preparation (mg/mL)
Acetonitrile	2
Chlorobenzene	1.7
Cumene	0.3
Cyclohexane	16
1,2-dichloroethene	9
Dichloromethane	3
1,4-dioxane	1.8
Methanol	14.5

Methylcyclohexane	5
Tetrahydrofuran	3.5
Toluene	4
Xylene	10.5

② Class 3A standard stock solution

Precisely weigh 250 mg each of acetone, ethyl acetate, diethyl ether, 2-propanol, isobutyl acetate, and 2-butanol and transfer to a 10 mL volumetric flask, respectively, add dimethyl sulfoxide to volume, and mix.

③ Class 2 and Class 3A standard solutions

Accurately take 1 mL of the Class 2A standard stock solution, transfer it in a 100 mL volumetric flask, add 1 mL of the Class 3A standard stock solution, and dilute with water to volume to prepare the standard stock solution (I). Put 1 mL of this solution in an appropriate headspace vial, add 5 mL of water. Apply the stopper, cap, and mix to prepare the standard solution (I).

b) Preparation of Class 2B and Class 3B standard solutions

① Class 2B standard stock solution

Dissolve chloroform, 1,2-dimethoxyethane, hexane, methyl butyl ketone, nitromethane, pyridine, tetralin, and 1,1,2-trichloroethene in dimethyl sulfoxide. Each solution is diluted as shown in the table below, and then is used as the Class 2B standard stock solution.

Solvent	Concentration of preparation (μg/mL)
Chloroform	60
1,2-dimethoxyethane	97
Hexane	186
Methylbutyl ketone	50
Nitromethane	49
Pyridine	200
Tetralin	97
1,1,2-trichloroethene	78

② Class 3B standard stock solution

Precisely weigh 50 mg of ethanol, acetone, t-butyl methyl ether, ethyl acetate, and 1-butanol, and transfer to a 10 mL volumetric flask, respectively, and dilute with dimethyl sulfoxide to volume.

③ Class 2B and Class 3B standard solutions

Take precisely 1 mL of the class 2B solvent standard stock solution, transfer it in a 100 mL volumetric flask, add 1 mL of the Class 3B solvent standard stock solution, and dilute with water to volume to prepare of the standard stock solution (II). Take 5 mL of this solution to an appropriate headspace vial, add 1 mL of water. Apply the stopper, cap, and mix to prepare the standard solution (II).

c) Standard solution by component

For the components detected following the Stage 1 and Stage 2 tests, dissolve each component RS in dimethyl sulfoxide by referencing the concentration of the standard stock solutions (I) and (II). Take precisely 1 mL of this solution, dilute it with water at each stage to a concentration similar to the concentration detected in the test solution during the Stage 1 and Stage 2 tests. If necessary, dilute it up to 20 times the concentration limit specified in the 「Guidelines for Residual Solvents in Pharmaceuticals」.

Transfer 1 mL of this solution to an appropriate headspace vial containing 5 mL of water. Apply the stopper, cap, and mix, and use it as the standard solution for each component.

2) Preparation of the test solution

a) Sample stock solution

Precisely weigh 250 mg of the sample, add water to make 25 mL, and use it as the sample stock solution.

b) Test solution

Transfer 5 mL of the sample stock solution to an appropriate headspace vial containing 1 mL of water. Apply the sopper, cap, and mix, and use it as the test solution.

c) Test solution containing the standard solution

Transfer 5 mL of sample stock solution to an appropriate headspace vial containing 1 mL of standard stock solution for each component. Apply the stopper, cap, and mix, and use it as the test solution containing the standard solution.

3) Gas chromatography operating conditions

Among the operating conditions, the internal diameter and length of the column, the particle size of the packing material, the concentration of the stationary phase, the column temperature and the flow rate of carrier gas may be modified within limits which allow the system suitability to be obtained. Additionally, the split ratio may be adjusted to obtain optimum sensitivity. The sample injection port and the operating conditions for headspace may also be modified within limits which allow for greater accuracy and precision more than those of a prescribed method to be achieved.

a) Method 1

Detector: Flame Ionization detector.

Column: 0.32 mm x 30 m fused silica column with a 1.8 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography. Or 0.53 mm x 30 m wide-bore column with a 3.0 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography.

Column temperature: Maintain the temperature at 30 °C for 20 minutes, then raise it to 240 °C at a rate of 10 °C per minute, and maintain it at 240 °C for 20 minutes.

Sample injection port temperature: 140 °C

Detector temperature: 250°C

Carrier gas: Helium or nitrogen

Flow rate: About 20 cm/sec

Split ratio: About 1:5

Conditions of sample injection outlet apparatus for headspace: It shall follow one of the following conditions.

	Condition 1	Condition 2	Condition 3
Equilibrium temperature (°C)	80	105	80
Equilibrium time (min)	60	45	45
Sample injection port and connector temperature (°C)	85	110	105
Headspace vial outlet temperature (°C)	80 – 90	105 – 115	80 – 90
Minimum pressurization time (seconds)	60	60	60

Injection volume (mL)	1	1	1

However, the order of elution for each component of the residual solvent is as follows.

1. **Solvents of Class 2A and Class 3A**

Order of elution	Residual solvent	Note
1	Methanol	Class 2
2	Diethyl ether	Class 3
3	Acetone	Class 3
4	2-Propanol	Class 3
5	Acetonitrile	Class 2
6	Dichloromethane	Class 2
7	*trans*-Dichloroethene	Class 2 1,2-Dichloroethene =*trans*-Dichloroethene + *cis*-Dichloroethene
8	*cis*-Dichloroethene	
9	Ethyl acetate	Class 3
10	Tetrahydrofuran	Class 2
11	2-Butanol	Class 3
12	Cyclohexane	Class 3
13	Methyl cyclohexane	Class 2
14	1,4-Dioxane	Class 2
15	Toluene	Class 2
16	Isobutyl acetate	Class 3
17	Chlorobenzene	Class 2
18	Ethyl benzene	Class 2 Xylenes = Ethyl benzene + *m*-Xylene + *p*-Xylene + *o*-Xylene
19	*m*, *p*-Xylene	
20	*o*-Xylene	
21	Cumene	Class 2

2. **Solvents of Class 2B and Class 3B**

Order of elution	Residual solvents (class 2B + 3)	Note
1	Ethanol	Class 3
2	Acetone	Class 3
3	Methyl *tert*-butylether	Class 3
4	Hexane	Class 2
5	Nitromethane	Class 2
6	Ethyl acetate	Class 3
7	Chloroform	Class 2
8	1,2-Dimethoxyethane	Class 2
9	1,1,2-Trichloroethene	Class 2
10	1-Butanol	Class 3
11	Pyridine	Class 2
12	Methylbutylketone	Class 2
13	Tetralin	Class 2

System suitability

Test for required detectability: In the system suitability solution, the signal-to-noise ratio of each peak is NLT 10.

System performance: When the test is repeated 6 times under the conditions shown above using the system suitability solution, the relative standard deviation of the peak area is NMT 10.0%, and the resolution from adjacent peaks is NLT 1.5. However, the resolution between the 2-propanol peak and the acetonitrile peak is NLT 1.0.

System suitability solution: At least 3 solvents, including the residual solvent to be analyzed, are selected and these are prepared in the same way as the standard solution.

b) Method 2

Detector: Flame Ionization detector.

Column: 0.32 mm x 30 m fused silica column with a 1.8 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography. Or, a 0.53 mm x 30 m wide-bore column with a 3.0 μm internal layer composed of 6% cyanopropylphenyl and 94% dimethylpolysiloxane gum for gas chromatography.

Column temperature: Maintain the temperature at 30 °C for 20 minutes, raise it to 240 °C at a rate of 10 °C per minute, and maintain it at 240 °C for 20 minutes.

Sample injection port temperature: 140 °C

Detector temperature: 250 °C

Carrier gas: Helium or nitrogen

Split ratio: About 1:5

Flow rate: About 20 cm/sec

Conditions of sample injection outlet apparatus for headspace: It shall follow one of the following conditions.

	Condition 1	Condition 2	Condition 3
Equilibrium temperature (℃)	80	105	80
Equilibrium time (min)	60	45	45
Sample injection port and connector temperature (℃)	85	110	105
Headspace vial outlet temperature (℃)	80 ~ 90	105 ~ 115	80 ~ 90
Minimum pressurization time (seconds)	60	60	60
Injection volume (mL)	1	1	1

However, the order of elution for each component of the residual solvent is as follows.

1. **Solvents of Class 2A and Class 3A**

Order of elution	Residual solvent	Note
1	Diethyl ether	Class 2
2	Cyclohexane	Class 3
3	Methyl cyclohexane	Class 2
4	Acetone	Class 3
5	Tetrahydrofuran	Class 2
5	*trans*-Dichloroethene	Class 2 1,2-Dichloroethene =*trans*-Dichloroethene + *cis*-Dichloroethene
6	Ethyl acetate	Class 3
7	Methanol	Class 2
8	Methylene chloride	Class 2
9	2-Propanol	Class 3
10	*cis*-Dichloroethene	Class 2 1,2-Dichloroethene =*trans*-Dichloroethene + *cis*-Dichloroethene
11	Acetonitrile	Class 2
12	Isobutyl acetate	Class 3
13	Toluene	Class 2
14	2-Butanol	Class 3
15	1,4-Dioxane	Class 2
16	Ethyl benzene	Class 2 Xylenes = Ethyl benzene + *m*-Xylene + *p*-Xylene + *o*-Xylene
17	*p*-Xylene	
18	*m*-Xylene	
19	Cumene	Class 2
20	o-Xylene	Class 2 Xylenes = Ethyl benzene + *m*-Xylene + *p*-Xylene + *o*-Xylene
21	Chlorobenzene	Class 2

2. Solvents of Class 2B and Class 3B

Order of elution	Residual solvent	Note
1	Hexane	Class 2
2	Methyl tert-butylether	Class 3
3	Acetone	Class 3
4	Ethyl acetate	Class 3
5	1,2-Dimethoxyethane	Class 2
6	Ethanol	Class 3
7	1,1,2-Trichloroethene	Class 2
8	Chloroform	Class 2
9	Methylbutylketone	Class 2
10	Nitromethane	Class 2
11	1-Butanol	Class 3
12	Pyridine	Class 2
13	Tetralin	Class 2

System suitability

Test for required detectability: In the system suitability solution, the signal-to-noise ratio of each peak is NLT 10.

System performance: When the test is repeated 6 times under the conditions shown above using the system suitability solution, the relative standard deviation of the peak area is NMT 10.0%, and the resolution from adjacent peaks is NLT 1.5. However, the resolution between p-xylene peak and *m*-xylene peak is NLT 1.0.

System suitability solution: At least 3 solvents, including the residual solvent to be analyzed, are selected and these are prepared in the same way as the standard solution.

4) Specification

Stage 1 Test

The test solution, standard solution (I), and standard solution (II) are tested using the gas chromatography method, following the gas chromatography operating conditions described in Method 1. The peak area is then calculated from each chromatogram. The peak area of each component in the test solution is smaller than the peak areas of each component in the standard solution (I) and standard solution (II).

Stage 2 Test

If, during the Stage 1 test, the peak area of the component in the test solution is equal to or greater than the peak area of each component in the standard solution (I) and standard solution (II), it is retested under the gas chromatography operating conditions described in Method 2. For the components identified when tested according to Method 1, the peak area of the component in the test solution is smaller than the peak area of the corresponding component in the standard solution when tested according to Method 2.

Stage 3 Test

If, during the Stage 2 test, if the peak area of the component in the test solution is equal to or greater than the peak area of the component in the standard solution, the detected components are tested under the gas chromatography operating conditions described in Method 1 using the test solution, the standard solution for each component, and the test solution containing the standard solution. The amount of residual solvent in the test solution is calculated from the peak areas A_T and A_{ST} in the test solution and the test solution containing the standard solution.

Amount of residual solvent in the test solution (ppm)

$$= \frac{(C \times n)}{W} \times \left[\frac{A_T}{(A_{ST} - A_T)}\right]$$

C: Concentration of standard solution (μg/mL)
W: Amount of sample taken (g)
n: Dilution factor from the standard stock solution

3. Solvents of Class 3

Perform the test using the test solution and standard solutions as described in gas chromatography. However, details necessary for testing, such as the amount of samples and reference standard collected, the preparation method, the amount of injection, the operating conditions of the headspace and the operating conditions of the gas chromatography, are specified in the monograph.

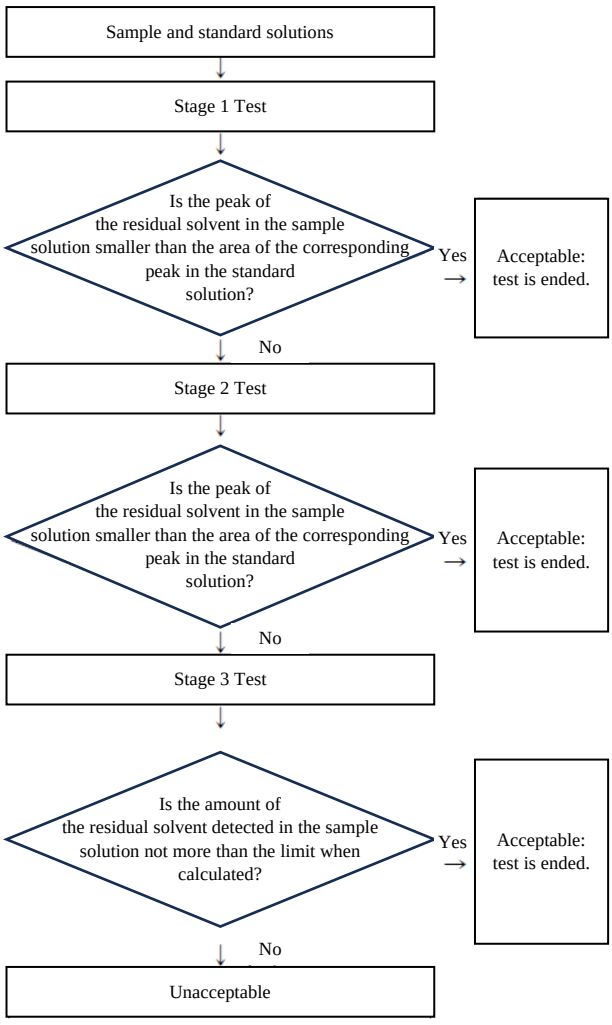


Figure 1. Test flow chart by stage and determination

Table 1. Classification of Residual Solvents

Class 1	Benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethane, 1,1,1-trichloroethane.
Class 2	Acetonitrile, chlorobenzene, chloroform, cumene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,2-dimethoxyethane, N,N-dimethylacetamide, N,N-dimethylformamide, 1, 4-dioxane, 2-ethoxyethanol, ethylene glycol, formamide,

	hexane, methanol, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidone, nitromethane, pyridine, sulfolane, tetrahydrofuran, tetralin, toluene, 1,1,2-trichloroethene, xylene[Note)]
Class 3	Acetic acid, acetone, anisole, 1-butanol, 2-butanol, n-butyl acetate, t-butyl methyl ether, dimethyl sulfoxide, ethanol, ethyl acetate, diethyl ether (ether), ethyl formate, formic acid, heptane, acetic acid Isobutyl, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methyl ethyl ketone, methyl isobutyl ketone, 2-methyl-1-propanol, pentane, 1-pentanol, 1-propanol, 2-propanol, propyl acetate

Note) In general, 60% m-xylene, 14% p-xylene, and 9% o-xylene containing 17% ethylbenzene

Residue on Ignition
강열잔분시험법

The Residue on Ignition is a method to measure the amount of the residual substances that are not volatilized when the sample is ignited according to the procedure described below.

This method is usually used to determine the content of inorganic substances contained as impurities in organic substances, but in some cases, it is used to measure the amount of impurities contained in organic substances or inorganic substances that volatilize when ignited.

For example, in the individual monograph, "NMT 0.10% (1 g)," indicates that when 1 g of this substance is precisely weighed and ignited according to the procedure described below, the mass of the residue is 1 mg or less per 1 g of this substance. In addition, when “after drying” is specified, the sample is collected after drying under the conditions specified in the Loss on Drying.

Procedure

Ignite a crucible made of materials such as platinum, quartz, and porcelain at 600 ± 50°for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccants), and weigh it accurately.

Place the amount of the sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at the lowest temperature possible, until the sample is thoroughly charred. After cooling, moisten the residue again with a small amount (usually 1 mL) of sulfuric acid, heat gently until no white vapor is generated, and ignite again at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not generated at any point during the procedure. Cool the crucible by placing it in a desiccator (silica gel or other suitable desiccants), and weigh it accurately to calculate the percentage of the residue.

If the percentage of the residue exceeds the limit specified in the monograph, the following is performed unless otherwise specified. Moisten the sample again with sulfuric acid, as described above, and heat and ignite the sample again for 30 minutes until the weight difference between the before and after is NMT 0.5 mg or when the percentage of the residue is NMT the limit specified in the monograph. The test is considered complete at this stage.

Safety
안전성시험법

The Safety is to detect toxic substances contained in drugs.

Test animals

Use healthy mice weighing 17 to 23 g.

Concentration and Dosage

The concentration of the sample is specified in the monograph. The solvent used in the preparation of the sample includes saline injection, water for injection, or as specified in the monograph. The dose of the sample is 0.5 mL per mouse unless otherwise specified.

Procedure

Unless otherwise specified, the specified amount of sample is injected into the tail vein of the 5 test animals with a 26 G needle for 15 to 30 seconds. Observe the test animals for 48 hours. If one test animal dies or NLT two test animals show abnormal symptoms, repeat the test using 10 mice weighing 20 ± 1 g.

Interpretation

A safety test result is considered suitable if no test animal dies and NMT one test animal shows abnormal symptoms. In addition, in the repeated test, the result is considered suitable if no animal dies and NMT one test animal shows abnormal symptoms.

Size-exclusion Chromatography
크기배제 액체크로마트그래프법

The Size-exclusion Chromatography is a liquid chromatography (2.2.29) technique that separates molecules in solution according to their size. When using organic mobile phases, the technique is referred to as gel-permeation chromatography, and with aqueous mobile phases, it is known as gel-filtration chromatography. The sample is introduced into a column, which is filled with a gel or a porous particle packing material, and is carried by the mobile phase through the column. The size separation takes place by repeated exchange of the solute molecules between the solvent of the mobile phase and the same solvent in the stagnant liquid phase (stationary phase) within the pores of the packing material. The pore-size range of the packing material determines the molecular-size range within which separation can occur.

Molecules small enough to penetrate all the pore spaces elute at the total mobile phase volume V_t also known as total permeation volume. In other words, the total mobile phase volume (V_t) is flown, and then small molecules eluted. Molecules apparently larger than the maximum pore size of the packing material migrate along the column only through the spaces between the particles of the packing material without being retained and elute at the retention volume of an unretained compound V_0 (also known as exclusion volume or void volume). In other words, the exclusion volume (V_0) of mobile phase is flown, and then large molecules eluted. Molecules of medium size elute between the total mobile phase volume (V_t) and the retention volume of an unretained compound (V_o). Separation according to molecular size occurs between the retention volume of an unretained compound and the total mobile phase volume, with useful separation usually occurring in the first two thirds of this range.

The apparatus consists essentially of a chromatographic column of varying length and internal diameter, if necessary temperature-controlled, packed with a separation material capable of fractionation in the appropriate range of molecular sizes. The eluent is passed through the column at a constant rate. One end of the column is usually fitted with a suitable device like an injection port for applying the sample and connected to a suitable pump for controlling the flow of the eluent. The outlet of the column is usually connected to a detector fitted with an automatic recorder which enables the monitoring of the relative concentrations of separated components of the sample.

Detectors typically utilize photometric, refractometric, or luminescent properties. An automatic fraction collector may be attached if necessary. The packing material may be a soft support, such as a swollen gel or a rigid support composed of a material such as glass, silica or a solvent-compatible, cross-linked organic polymer. Rigid supports usually require pressurized systems for faster separations. The mobile phase is chosen according to sample type, separation medium and method of detection.

Before carrying out the separation, the packing material is treated, and the column is packed as described in the monograph or according to the manufacturer's instructions. The chromatographic system is assessed for the system suitability as described under the Chromatography.

Determination of relative component composition of mixtures

Perform the separation process according to the regulations of each pharmacopoeial monograph. If the sample is monitored by a physico-chemical property to which all the components of interest exhibit equivalent responses (for example if they have the same specific absorbance), calculate the relative amount of each component by dividing the respective peak area by the sum of the peak areas of all the components of interest. However, if the concentrations of the physicochemical property used for detection vary among the components of interest, calculate the content using calibration curves derived from the standards specified in the monograph.

Determination of molecular masses

Size-exclusion chromatography may be used to determine molecular masses by comparing them with appropriate calibration standards specified in the monograph. The retention volumes of the calibration standards may be plotted against the logarithm of their molecular masses. The plot usually approximates a straight line within the exclusion and total permeation limits for the separation medium used. Molecular masses can then be determined using the calibration curve. It is important to note that the validity of the molecular mass calibration is limited to the particular macromolecular solute/solvent system used under the specified experimental conditions.

Determination of molecular size distribution of polymers

Size-exclusion chromatography can be used to assess the distribution of the molecular sizes of polymers. However, the comparability of samples is only assured for results obtained under identical experimental conditions. The reference standards used for the calibration and the methods for determination of the distribution of molecular sizes of polymers are specified in the monograph.

Specific Gravity and Density
비중 및 밀도측정법

The density ρ (g/mL or g/cm^3) is the mass per unit volume, and the specific density *d* is the ratio of the mass of a substance with a certain volume to the mass of a standard substance with the same volume. It is also known as relative gravity. The specific gravity $d_t^{t'}$ refers to the ratio of the mass of the sample at t′ °C to that of water (H_2O) with the same volume at t °C. Unless otherwise specified, the measurement should be performed using Method 1, Method 2, or Method 4. When the measurement value is accompanied by the term "about", Method 3 can be used.

Method 1. Assay by a pycnometer

A pycnometer is a glass vessel usually with a capacity of 10 mL to 100 mL, which includes a ground-glass stopper fitted with a thermometer and a side tube with a gauge line and a ground-glass cap. Weigh a pycnometer, cleaned and dried in advance, and refer to its mass as M. Remove the stopper and the cap and fill the pycnometer with the sample. At a temperature 1 °C to 3 °C lower than the specified temperature t′ °C, close the stopper, taking precautions to avoid leaving any bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the sample above the gauge line from the side tube, close the side tube cap, and wipe the outside surface thoroughly. Measure the mass M1 of the pycnometer.

Fill the same pycnometer with water to perform the same procedure. Measure the mass M_2 at the specified temperature *t* °C and calculate the specific gravity $d_t^{t'}$ using the following formula.

$$d_t^{t'} = \frac{M_1 - M}{M_2 - M}$$

When measuring with the sample and water at the same temperature (*t*′= *t*), the density of the sample at the temperature $\rho_T^{t'}$ can be calculated from the measured specific gravity $\rho_{S1}^{t'}$ and density of water at the temperature t′ °C $d_t^{t'}$ indicated in the Table using the following formula:

$$\rho_T^{t'} = \rho_{S1}^{t'} \cdot d_t^{t'}$$

Table. Density of water

Temperature ℃	Density g/mL	Temperature ℃	Density g/mL	Temperature ℃	Density g/mL	Temperature ℃	Density g/mL
0	0.99984	10	0.99970	20	0.99820	30	0.99565
1	0.99990	11	0.99961	21	0.99799	31	0.99534
2	0.99994	12	0.99950	22	0.99777	32	0.99503
3	0.99996	13	0.99938	23	0.99754	33	0.99470
4	0.99997	14	0.99924	24	0.99730	34	0.99437
5	0.99996	15	0.99910	25	0.99704	35	0.99403
6	0.99994	16	0.99894	26	0.99678	36	0.99368
7	0.99990	17	0.99877	27	0.99651	37	0.99333
8	0.99985	18	0.99860	28	0.99623	38	0.99297
9	0.99978	19	0.99841	29	0.99594	39	0.99259
10	0.99970	20	0.99820	30	0.99565	40	0.99222

Method 2. Assay by a Sprengel Ostwald

Pycnometer A Sprengel-Ostwald Pycnometer is a glass vessel usually with a capacity of 10 mL to 100 mL. As shown in

Figure 1, both ends of the vessel are thick-walled fine tubes (internal diameter: 1 mm - 1.5 mm, outside diameter: 3 mm - 4 mm), and Tube A on one end has a gauge line C. Weigh a pycnometer, cleaned and dried in advance, to determine its mass M by hanging it on the ring of a chemical balance beam with a wire D made of platinum, aluminum, etc. Immerse the fine tube B in the sample, which is at 3 °C to 5 °C lower than the specified temperature. Attach a rubber tube or a ground-glass column to the end of A, and suck up the sample up to the above-gauge line C, taking precautions to prevent the formation of bubbles. Immerse the pycnometer on a water bath at a specified temperature t' °C for about 15 minutes. Then, adjust the sample level to the gauge line C by attaching a piece of filter paper to the end of B. Take the pycnometer out of the water bath, wipe the outside surface thoroughly, and weigh the mass M_1. Fill the water into the same pycnometer and repeat the same procedure. Measure the mass M2 at the specified temperature t °C. Calculate the specific gravity $d_t^{t'}$ according to the formula described in Method l. When measuring with the sample and water at the same temperature ($t' = t$), calculate the density $\rho_T^{t'}$ of the sample at the temperature t′ °C by using the formula described in Method l:

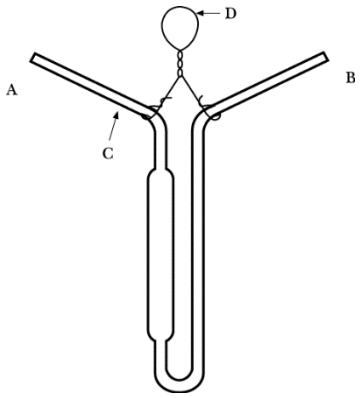


Figure 1. Sprengel-Ostwald pycnometer.

Method 3. Assay by a hydrometer

Clean a hydrometer with ethanol or ether. Stir the sample well with a glass rod and add a hydrometer. When the hydrometer comes to a standstill at the specified temperature t' °C, read the specific gravity $d_t^{t'}$ or the density $\rho_T^{t'}$ at the upper brim of the meniscus. Here, t' °C indicates the temperature at which the hydrometer is calibrated. In addition, if there are specific read instructions indicated on the hydrometer, follow the instructions. And, when the temperature t' °C when the sample is measured is equal to the temperature when the meniscus is calibrated ($t' = t$), calculate the density of the sample at the temperature t' °C from the specific gravity $d_t^{t'}$ using the formula shown in Method l:

Method 4. Assay by an oscillator-type density meter

Density measurement with an oscillator-type density meter obtains the density of a sample by measuring a natural frequency T (s) of a glass column cell containing the liquid or gas sample. When providing vibration to a sample cell containing the liquid or gas sample to be measured, it vibrates with an intrinsic oscillation period depending on the mass of the sample. If the volume of the vibrating part of the sample cell is kept constant, there is a linear relationship between the square of the intrinsic oscillation period and the density of the sample. To measure the density of a sample using this method, the intrinsic oscillation period: T_{S1}, T_{S2} of two standard materials (density:ρ_{S1}, ρ_{S2}) must each be measured at a specified temperature t' °C, and the sample cell constant $K_{t'}$ (g·cm^{-3}s^{-2}) must be determined using the following formula:

$$K_{t'} = \frac{\rho_{S1}^{t'} - \rho_{S2}^{t'}}{T_{S1}{}^2 - T_{S2}{}^2}$$

Usually, water and dried air are used as standard materials. Here, the density of water at t' °C, $\rho_{S1}^{t'}$ is obtained from the Table, and that of dried air, $\rho_{S2}^{t'}$ is calculated using the following formula, where the pressure of dried air is set to pkPa.

$$\rho_{S2}^{t'} = 0.0012932 \times \{ 273.15 / (273.15 + t') \} \times (p / 101.325)$$

Next, add a sample into a sample cell for which the cell constant is known. Measure the sample's intrinsic oscillation period T_T by repeating the same procedure, then the density $\rho_T^{t'}$ of the sample can be calculated using the previously calculated intrinsic oscillation period T_{S1} and the density of water $\rho_{S1}^{t'}$ at the specified temperature t' °C.

$$\rho_T^{t'} = \rho_{S1}^{t'} + K_{t'}(T_T{}^2 - T_{S1}{}^2)$$

Calculate the specific gravity of a sample $d_t^{t'}$ to water at a temperature t °C according to the following formula by using the density of water $\rho_{S1}^{t'}$ at a temperature t °C in the Table.

$$d_t^{t'} = \frac{\rho_T^{t'}}{\rho_{S1}^{t'}}$$

Apparatus

An oscillator-type density meter is usually composed of a glass column cell with a capacity of about 1 mL, where the curved end is fixed to the vibration reed; an oscillator which applies an initial vibration to the cell; a detector for measuring the intrinsic oscillation period; and, a temperature controlling system. A schematic illustration of the apparatus is depicted in the Figure.

Procedure

Adjust a sample cell, water, and a sample to a specified temperature t' °C. Wash the sample cell with water or an appropriate solvent and dry it thoroughly with a flow of dried air. Stop the flow of dried air and confirm that the temperature is kept at the specified value. Then, measure the intrinsic oscillation period T_{S2} of the dried air. Separately measure the atmospheric pressure p (kPa). Next, add water into the sample cell and measure the intrinsic oscillation period T_{S1} of water. Using these values of the intrinsic oscillation period for water and air, determine the sample cell constant $K_{t'}$. Next, add a sample into the glass cell, confirm that the temperature is kept at the specified value, and measure the intrinsic oscillation period T_T given by the sample. Using the intrinsic oscillation periods of water and sample, the density of water $\rho_{S1}^{t'}$, and the cell constant $K_{t'}$, calculate the density of the sample $\rho_T^{t'}$. If necessary, the specific gravity of the sample $d_t^{t'}$ to water at a temperature t °C can be calculated using the density of water $\rho_{S1}^{t'}$ shown in the Table. Take care to prevent the formation of bubbles in the sample cell when a sample or water is added into the cell.

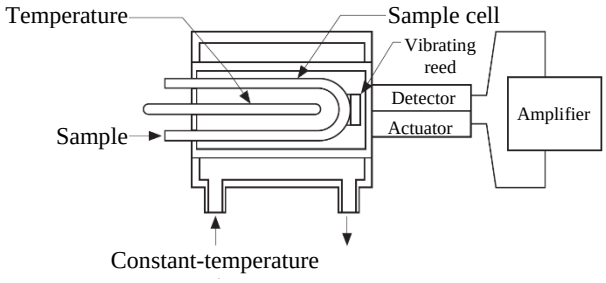


Figure 2. An oscillator-type density meter

Sterility
무균시험법

The Sterility is applied to pharmaceutical ingredients, preparations or articles which, according to the Pharmacopeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganisms were detected in the sample examined under these test conditions.

Precautions against microbial contamination

The test for sterility is performed under aseptic conditions. Therefore, the test environment should be adapted to perform the sterility test. The precautions taken to avoid contamination should not affect any microorganisms detected by this test. The test workspace should be monitored regularly through appropriate sampling and controls.

Culture media and incubation temperatures

1) General requirement

The media for the test are prepared as follows, or an equivalent commercial medium may be used if it is suitable for the growth promotion test. Suitable media for the sterility test are as follows. A fluid thioglycollate medium is primarily used to culture the anaerobic bacteria, but also can detect the aerobic bacteria. Soybean casein digest medium is suitable for the cultivation of fungi and aerobic bacteria.

2) Fluid thioglycollate medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose	5.0 g
or Glucose monohydrate	5.5 g
Yeast extract (water-soluble)	5.0 g
Casein peptone (pancreatic digest of casein)	15.0 g
Sodium thioglycollate	0.5 g
or Thioglycolic acid	0.3 mL
Resazurin solution (1 in 1000), freshly prepared	1.0 mL
Water	1000 mL
(pH after sterilization: 7.1 ± 0.2)	

Mix L-cystine, agar, sodium chloride, glucose, yeast extract and casein peptone (pancreatic digest of casein) with water and heat to dissolve. Then, dissolve in sodium thioglycolate or thioglycolic acid, add 1 mol/L sodium hydroxide TS if necessary, sterilize it, and adjust the pH to 7.1 ± 0.2. If necessary, heat the solution again without boiling, and filter it through a moistened filter paper while still hot. Add resazurin solution (1 in 1000) and mix well. When the culture is finished, transfer the required amount to a vessel with a surface-to-depth ratio that allows the pink-colored part of the medium to be less than 1/2 the distance from the top, and sterilize it using a validated process. If it is necessary to store the medium, put it in a tightly sealed container, previously sterilized, and store it at 2 to 25 ℃. When more than the upper 1/3 of the medium turns pink in color, heat the medium container on a water bath or free-flowing steam until the pink color disappears, and cool it rapidly, taking care to prevent the introduction of non-sterile air into the container. It can be used only once. Media that have exceeded the validation period should not be used.

Incubate the fluid thioglycollate medium at 30 – 35 ℃. Products containing mercury-based preservatives, for which the membrane filter method cannot be used, can be cultured at 20 – 25 °C using the fluid thioglycollate medium instead of the soybean casein digest medium, if it has been validated as described in the growth promotion test. In cases specified otherwise, a modified thioglycollate medium prepared as follows may also be used. Prepare the same composition as the fluid thioglycollate medium except for agar and resazurin solution (1 in 1000) and sterilize it using a validated process. Adjust the pH to 7.1 ± 0.2 after sterilization and heat it on a water bath immediately before use. Incubate the modified thioglycolate medium at 30 – 35 ℃ under anaerobic conditions.

3) Soybean casein digest medium

Casein peptone (pancreatic digest of casein)	17.0 g
Soybean peptone (papaic digest of soybean meal)	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate	2.5 g
Glucose	2.3 g
or Glucose monohydrate	2.5 g
Water	1000 mL
(pH after sterilization: 7.3 ± 0.2)	

Dissolve all ingredients in water and warm it slightly to make a solution. Cool the solution to ordinary temperature, add 1 mol/L sodium hydroxide TS if necessary, sterilize it, and adjust the pH to 7.3 ± 0.2. Filter, if necessary, divide in the required amount into suitable vessels, and sterilize it using a validated process. If it is not going to be used immediately, store at 2 – 25 °C in a sterile tight container. Any media that have exceeded the validation period should not be used. Incubate the soybean casein digest medium at 20 – 25 °C.

Suitability of culture media

The media comply with the following tests. These tests can be performed before or in parallel with the sterility test of the product.

1) Sterility of the medium

No growth of microorganisms should be observed when a portion of the media is incubated for 14 days.

2) Growth promotion test for aerobic bacteria, anaerobic bacteria, and fungi

Test each batch of ready-prepared medium and dehydrated medium or medium prepared with each ingredient. Suitable strains of microorganisms are indicated in Table 1.

Inoculate portions of fluid thioglycollate medium with a small number (NMT 100 CFU) of the following microorganisms, using a separate medium for each of the following species of microorganism:

Clostridium sporogenes, *Pseudomonas aeruginosa*, *Staphylococcus aureus.*

Inoculate portions of soybean casein digest medium with a small number (NMT 100 CFU) of the following microorganisms, using a separate medium for each of the following species of microorganism:

Aspergillus brasiliensis, *Bacillus subtilis*, *Candida albicans*

Incubate bacteria for NMT 3 days and fungi for NMT 5 days.

Test with a seed lot system for the management of inoculums to ensure that they are NMT 5 passages removed from the master seed lot. The medium is suitable when the growth of microorganisms is clearly visible.

Table 1. Strains of the test microorganisms suitable for use in the growth promotion test and the method suitability test

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276, KCTC 3881※
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134, KCTC 1021※
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275, KCTC 2513※
Anaerobic bacterium	
Clostridium sporogenes	ATCC 19404, CIP 79.3, NCTC 532, or ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594, KCTC 7965※
Aspergillus brasiliensis (Aspergiluus Niger)	ATCC16404, IP 1431.83, IMI 149007, NBRC 9455, KCTC 6317 or 6196※

※ Strains of the test microorganisms validated by the Korean Collection for Type Cultures (KCTC)

Method suitability test

Except for the modifications described below, perform the test in the same manner as the 『Sterility test of the product to be examined』.

1) Membrane filtration

After filtering the contents of the container to be tested, add NMT 100 CFU of strains of the test microorganisms to the final portion of the sterile diluent used to rinse the filter, and filter it.

2) Direct inoculation

Transfer the contents of the container to be tested into a medium and inoculate NMT 100 CFU of the strains of the test microorganisms to the medium. In both cases, use the same microorganisms described above under 2) Growth promotion test for aerobic bacteria, anaerobic bacteria, and fungi in 'Suitability of culture media'.

Perform a test under 2) Growth promotion test in 'Suitability of culture media' as a positive control group.

Incubate all containers containing the medium for NMT 5 days at the specified temperature. If there is a clearly visible growth equivalent to the positive control group after incubation when observed with the naked eye, the sample is considered to have no antibacterial activity, or that it was satisfactorily eliminated under these test conditions. This method is suitable as a sterility test and does not require any modifications to the test conditions. If a clearly visible growth equivalent to the positive control group cannot be seen, the antibacterial activity of the sample is not sufficiently eliminated under these test conditions. In this case, modify the conditions to eliminate the antibacterial activity and repeat the method suitability test.

This method suitability test is performed:

A) when the sterility test is performed for a new product.

B) whenever any test conditions are changed.

The method suitability test may be performed simultaneously with the Test for sterility of the product to be examined.

Test for sterility of the product to be examined

1) General requirements

Perform the test with the membrane filtration method or the direct inoculation. Appropriate negative controls are included. The technique of membrane filtration is applied to the filterable products. For example, it is filterable aqueous, alcoholic, or oily products and products miscible with or soluble in aqueous or oily solvents without any antibacterial effect that are tested under this condition.

2) Membrane filtration

Use membrane filters with a nominal pore size no greater than 0.45μm which have been proven to be effective in retaining microorganisms. For example, cellulose nitrate filters are used in aqueous, oily, and weakly alcoholic solutions, while cellulose acetate filters are used in strong alcoholic solutions. Specially adapted filters may be required for certain products, such as antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters with a different diameter are used, the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and the membrane filter are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions, it allows aseptic removal of the membrane filter for inoculating to the medium, or is suitable for performing the incubation after adding the medium to the apparatus itself.

A) Aqueous solutions

Transfer a small quantity of a suitable sterile diluent, such as 1 g/L neutral solution of meat or casein peptone (pH 7.1 ± 0.2), to the membrane filter and filter it. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container(s) to be tested to the membrane(s), after diluting to the volume used in the method suitability test with the chosen sterile diluent if necessary but in any case using no less than the quantities of the product to be examined prescribed in Table 2. Filter immediately. If the product has antibacterial properties, wash the membrane filter at least 3 times with the amount of sterile diluent used in the method suitability test. Even if the antibacterial activity was not sufficiently removed in the method suitability test, do not perform more than 5 washing cycles with 100 mL per membrane filter. Remove the membrane filter from the filtration apparatus, transfer the whole membrane filter to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Alternatively, transfer the medium into the apparatus with membrane filters. The amount of each medium is the same as the amount used in the method suitability test. Incubate the media for at least 14 days.

Table 2. Minimum quantity to be used for each medium

Quantity per container	**Minimum quantity to be used for each medium unless otherwise specified and authorized**
Liquids	

Less than 1 mL	Total amount
1 to 40 mL	Half, but 1 mL or more
Greater than 40 mL, and not greater than 100 mL	20 mL
Greater than 100 mL	10%, but 20 mL or more
Antibiotic liquids	1 mL
Insoluble preparations, creams, and ointments to be suspended or emulsified	0.2 g or more
Solids	
Less than 50 mg	Total amount
50 mg or more and less than 0.3 g	Half, but 50 mg or more
0.3 g or more and less than 5 g	0.15 g
Greater than 5 g	0.5 g

B) Soluble solids

Use each medium in no less than the quantity indicated in Table 2. Dissolve in an appropriate solvent, such as the solvent provided for the preparation, water for injection, saline injection, or 1 g/L neutral solution of meat or casein peptone. Use a membrane filter appropriate for the selected solvent and perform the test according to A) Aqueous solutions.

C) Oils and oily solutions

Use no less than the amount indicated in Table 2 for each medium. Oils and oily solutions with sufficiently low viscosity are filtered through a dry membrane filter without dilution. Viscous oils may be diluted with a suitable sterile solvent, such as isopropyl myristate, which has been proven to have no antibacterial properties under the corresponding test conditions. Allow the oil to penetrate the membrane by its own weight and then filter, applying the pressure or suction gradually. Wash the membrane filter 3 times with about 100 mL of an appropriate sterile solution, such as 1 g/L neutral solution of meat or casein peptone containing an appropriate emulsifier at a concentration that has been proven to be suitable in the method suitability test, e.g., polysorbate 80 at a concentration of 10 g/L. As described in A) Aqueous solutions, place a membrane filter in the medium, or add the medium to the filter and incubate at the same temperature for the same period of time.

D) Ointments and creams

Use each medium in no less than the quantity indicated in Table 2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted with 1% isopropyl myristate as described above. If necessary, heat the product at 40 °C or less. As an exception, it may be heated at 44 °C or less. Filter as rapidly as possible and perform the test according to C) Oil and oily solutions.

3) Direct inoculation of the culture medium

Transfer the quantity of the preparation to be examined prescribed in Table 2 directly into the culture medium so that the volume of the product is NMT 10% of the volume of the medium, unless otherwise prescribed. If the product has antibacterial activity, neutralize it with an appropriate neutralizing agent or dilute it with a sufficient quantity of the medium before testing. When it is necessary to use a large volume of the product, it may be desirable to use a concentrated medium, considering the dilution effect caused by inoculation. Where appropriate the concentrated medium may be added directly to the product in its container.

A) Oily liquids

Use a medium to which a suitable emulsifying agent has been added at a concentration proven to be appropriate in the method suitability test, for example, polysorbate 80 at a concentration of 10 g/L.

B) Ointments and creams

Emulsify with the chosen emulsifying agent in a suitable sterile diluent such as 1 g/L neutral solution of meat or casein peptone, dilute with a sterile diluent (1 in 10), and inoculate to a medium not containing an emulsifying agent. Incubate the inoculated media for at least 14 days. Observe the condition of the cultures several times during the incubation period. Gently shake cultures containing oily product daily. However, when using the fluid thioglycollate medium to detect anaerobic bacteria, minimize shaking to maintain anaerobic conditions.

Observation and interpretation of results

During the incubation period and on the last day, visually examine the growth of the microorganisms in the medium. If the microbial growth cannot be readily determined through visual examination since the material being tested renders the medium turbid, inoculate a portion of the medium (NLT 1 mL) to a new container containing same medium after 14 days from the start of culture. Then, incubate the original medium and the inoculated medium for at least 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test is considered invalid in one or more the following circumstances:

A) The data of the microbiological monitoring of the sterility testing facility show a fault;

B) A review of the testing procedure used during the sterility test in question reveals a fault;

C) Microbial growth is observed in the negative controls;

D) After identifying the microorganisms isolated from the sterility test, it is determined clearly that the growth of this (or these) species can be ascribed unequivocally to faults in the material and/or technique used in conducting the sterility test procedure.

If the test is declared to be invalid, repeat the test with the same number of units as the first test. If no evidence of microbial growth is found during the retest, the product to be examined complies with the test for sterility. If microbial growth is observed during the retest, the product examined does not comply with for the test for sterility.

Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

When using the membrane filtration technique, use the entire contents of the container if possible. However, use more than the quantities indicated in Table 2. If necessary, dilute it with about 100 mL of an appropriate sterile solution, such as 1 g/L meat or casein peptone neutral solution.

When using the technique of direct inoculation of media, use the quantities indicated in Table 2 unless otherwise specified. The sterility tests for bacteria and fungi are performed on the same sample of the product to be examined. If the volume or quantity in a single container is insufficient to perform the tests, the contents of 2 or more containers are used to inoculate the different media.

Minimum number of products to be tested

The minimum number of products to be tested is indicated in Table 3 according to the lot size.

Table 3. Minimum number of products to be tested

Number of samples per the batch*	Minimum number of samples to be tested for each medium, unless otherwise specified and authorized**
Parenteral preparations	
NMT 100 containers More than 100 but NMT 500 containers More than 500 containers	10% or 4 containers, whichever is the greater 10 containers 2% or 20 containers (10 containers large-volume preparations), whichever is less
Ophthalmic and other non-injectable preparations	
NMT 200 containers More than 200 containers	5% or 2 containers, whichever is the greater 10 containers
If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral administration.	
Bulk solid products	
Up to 4 containers More than 4 containers but NMT 50 containers More than 50 containers	Each bulk container 20% or 4 containers, whichever is the greater 2% or 10 containers, whichever is the greater

* If the batch size is not known, use the maximum number of samples indicated.
** If the contents of one container are enough to inoculate the 2 media, use the number of containers indicated.

Sulfate
황산염시험법

The Sulfate is to test a limit for sulfate contained in drugs.

In the monograph, the permissible limit for sulfate (as SO4) is described in percentage(%) in parentheses.

Procedure

Unless otherwise specified, place the amount of the sample specified in the monograph into a Nessler tube, dissolve it in an appropriate amount of water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Separately, transfer a volume of 0.005 mol/L sulfuric acid VS, as specified in the monograph, to another tube, and use this solution as the blank. When the test solution is not clear, filter both solutions under the same conditions.

Add 2 mL of barium chloride TS to the test solution and the reference solution, mix well, and allow to stand for 10 minutes. Compare the turbidity produced in both solutions against a black background by observing the Nessler tube downward or transversely.

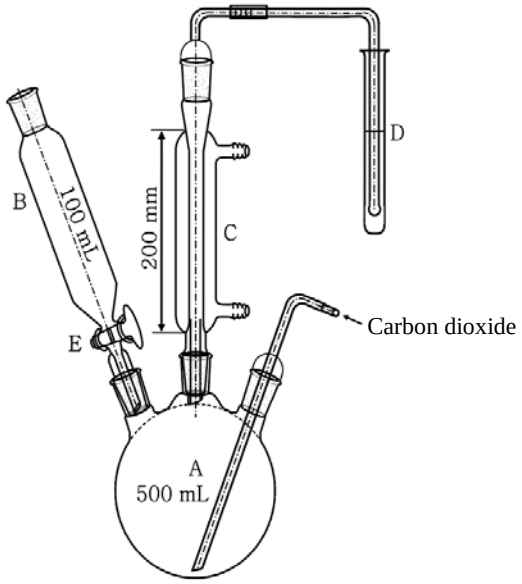
The turbidity produced in the test solution is not thicker than the turbidity produced in the control solution.

Sulfur Dioxide
이산화황시험법

This method consists in obtaining sulfur dioxide (SO2) by heating and distilling a sample in an acidic solution, collect and oxidize it in a hydrogen peroxide solution to obtain oxidized sulfuric acid, and titrate it with a sodium hydroxide solution.

Apparatus

Use the apparatus shown in the Figure.



A: Three-neck distilling flask
B: Cylindrical separatory funnel
C: Condenser D: Test tube
E: Stopcock

Procedure

Add 150 mL of water to a three-neck distilling flask (A), close the stopcock (E) of the cylindrical separatory funnel (B), and pass carbon dioxide through the whole device at a flow rate of 100 ± 5 mL/min. Pour cooling water into the cooler (C) and add 10 mL of dilute hydrogen peroxide solution TS into the test-tube (D). After 15 minutes, without interrupting the stream of carbon dioxide, remove the funnel (B) from the three-neck distilling flask (A), accurately weight about 25.0 g of the sample and transfer it to the three-neck distilling flask (A) using 100 mL of water. Apply coke lubricant to the outer surface of the connection part of the cylindrical separatory funnel (B) and install the cylindrical separatory funnel in its original position on the three-necked distilling flask. Close the cock (E) of the cylindrical separatory funnel, pour 80 mL of 2 mol/L hydrochloric acid solution into it, then open the tap of the funnel to allow the hydrochloric acid solution to flow into the flask, making sure that no sulfur dioxide escapes into the funnel by closing the tap before the last few mL of hydrochloric acid solution drains out. Boil for 1 hour. Transfer the contents of the test-tube(D) with the aid of a little water to a wide-necked Erlenmeyer flask. Heat it on a water bath for 15 min and allow to cool. Add 0.1 mL of a 1 g/L bromphenol blue TS and titrate with 0.1 mol/L sodium hydroxide solution until the color changes from yellow to violet-blue (V1) which lasts for 20 seconds. Perform a blank titration (V2) and correct using the same manner.

$$\text{Amount of sulfur dioxide (ppm)} = V / M \times 1000 \times 3.203$$

m = Mass of the sample (g)
V = Molarity of 0.1 mol/L sodium hydroxide solution used

as titrant (mL)

Thermal Analysis
열분석법

The Thermal Analysis is an analytical method used to measure the change in a physical property of a substance as a function of temperature. The most commonly used method is to measure changes in the mass or energy of a sample substance.

This method can be used in various applications:

- Determination of phase transitions
- Determination of changes in chemical composition
- Determination of purity

Of the above measurements, thermogravimetry (TG) can be used as "Loss on Drying" or "Water Determination." However, when TG is used as "Water Determination," it must first be confirmed that no volatile component except water is in the sample.

1. Method 1. Thermogravimetry

Thermogravimetry (TG) or thermogravimetric analysis (TGA) is used to measure the mass of a sample substance as a function of temperature according to a temperature control program.

1.1. Apparatus

The basic components of an apparatus for TG are a device for heating or cooling the substance according to a temperature control program, a sample holder in which the test conditions can be controlled, an electronic balance, and a device or computer for recording an electronic signal output.

1.2. Temperature calibration

A temperature sensor that is close to or in contact with the sample is calibrated using the Curie temperature of a ferromagnetic substance such as nickel. For an apparatus that performs TG/TGA and differential thermal analysis (DTA) or differential scanning calorimetry (DSC) at the same time, the same reference standards as those for DTA and DSC may be used (e.g., indium, tin, and zinc).

1.3. Calibration of electronic balance

Place an appropriate amount of calcium oxalate monohydrate RS or suitable certified reference standards in the sample holder and measure the mass. Set the heating rate according to the manufacturer's instructions (e.g., 5 °C/min) and increase the temperature. Create the thermogravimetric graph by plotting temperature or time on the horizontal axis (increasing from left to right) and mass on the vertical axis (decreasing downward). Stop increasing the temperature at about 250 °C. Measure the difference between the initial and final mass-temperature or mass-time plateau on the graph. this difference corresponds to a mass loss. The mass loss of the certified reference standard uses theoretical values.

1.4. Method

Perform the test with a sample substance according to the conditions specified in the monograph. Calculate the decrease in the mass of the sample using the difference determined by the graph obtained in the test and indicate the decrease in the mass in $\Delta m/m(\%)$. If the apparatus is used frequently, perform temperature calibration regularly. Otherwise, perform calibration before each measurement. Since the operating conditions are critical, check the following parameters for each measurement: pressure or flow rate, gas composition, mass of the sample, heating rate, temperature range, and sample pretreatment including isothermal phase, etc.

2. Method 2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a method used to detect the energy phenomena that occur when a substance (or a mixture of substances) is heated (or cooled) and to measure the temperature change, such as enthalpy or specific heat.

This method is used to determine the difference in heat flux (with respect to the temperature) generated or absorbed by the sample compared with the reference cell as a function of the temperature. There are two types of DSC apparatus: power-compensated differential scanning calorimetry (power-compensated DSC), which attempts to keep the temperature difference between the sample and reference at zero, and heat flux differential scanning calorimetry (heat flux type DSC), which detects the temperature difference as the difference in heat flux between the sample and reference when heated at a constant rate.

2.1. Apparatus

The power-compensated DSC apparatus has a furnace containing a sample vessel with a reference cell and a sample cell. The heat-flux DSC apparatus has a furnace containing a single cell with a sample holder for a reference cell and a sample cell. It also has a temperature control programming device, heat detector(s), and a recording device that can be connected to a computer, and measurements are performed under controlled conditions.

2.2. Calibration of apparatus

Calibrate the apparatus for temperature and enthalpy changes using certified reference standards.

2.2.1. Temperature calibration

Calibration of the temperature is performed using certified reference standards with an intrinsic thermal property, such as the melting point of metals or organic substances of high purity, or the phase transition point of crystalline inorganic salts or oxides. Normally, the melting points of indium, tin, and zinc are used for calibration.

2.2.2. Calibration of the calorimetry

To accurately estimate the calorimetric change (enthalpy change) of a sample based on physical change accompanied by temperature change, it is necessary to calibrate the apparatus using suitable certified reference standards. As in 2.2.1. Temperature Calibration, calibration of the quantity of heat can be performed using suitable certified reference standards that exhibit a constant enthalpy change caused by physical changes, such as the melting of pure metals and/or organic substances or the phase transition of crystalline inorganic salts. Normally, the melting points of indium, tin, and zinc are used for calibration.

2.3. Procedure

Weigh the appropriate amount of the sample into the suitable container and place it in the sample holder. Place an empty container in the reference holder. Set the initial and final temperatures and the heating rate according to the operating conditions specified in the corresponding monograph. Start the analysis and measure the DSC curve by plotting temperature or time on the abscissa (increasing from left to right) and the energy change on the ordinate (specify whether the change is an endothermic or an exothermic change).

The temperature at which the phenomenon occurs (the initial temperature) corresponds to the intersection of the tangent with the maximum slope (inflection point) in the curve and the extension of the baseline (A in Figure 1).
The endpoint of the thermal event is indicated by a peak on the curve.

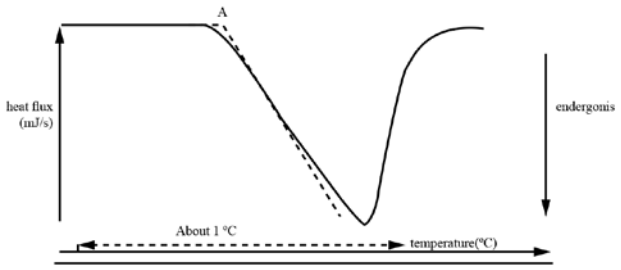


Figure 1. Thermogram

The enthalpy of this phenomenon is proportional to the area under the curve surrounded by the baseline. The proportionality factor is determined by measuring the heat of fusion of a known substance (e.g., indium) under the same operating conditions.

Label each thermogram with the following information: experimental conditions, record of last calibration, amount and type of the sample (including thermal history), container, measurement conditions (type, flow rate, pressure), direction and rate of temperature change, sensitivity of the apparatus and the recorder.

2.4. Applications

2.4.1. Phase transition

As a function of temperature, the transitions shown in Table 1 below can be observed: the temperature of the phase change occurring in the sample, the change in heat capacity, and the enthalpy change.

Table 1

Solid-solid transition	Allotropy-polymorphism
	Desolvation
	Amorphous-crystalline
Solid-liquid transition	Melting
	Glass transition
Solid-gas transition	Sublimation
Liquid-solid transition	Freezing
	Recrystallization
	Glass transition
Liquid-gas transition	Evaporation

2.4.2. Changes in chemical composition

By measuring the heat of reaction and reaction temperature under given test conditions, changes in chemical composition can be determined, such as the rate of a decomposition or desolvation reaction, for example.

2.4.3. Application to phase diagrams

DSC can be used to plot phase diagrams for solid mixtures. The preparation of a phase diagram is an important step in the preformulation and optimization of the lyophilization process.

2.4.4. Determination of purity

It is possible to determine the content of impurities in a substance from a single thermogram by measuring the proportion of a substance that melts at a specific temperature. There is no need to repeatedly and accurately measure the true value of the temperature, and only a sample of several mg is required to determine the content of impurities. Theoretically, the melting of a pure crystalline substance at constant pressure is characterized by an extremely narrow range of heat of fusion ΔH_f corresponding to the melting point T_0. A broadening of the melting temperature range is a sensitive indicator of impurities. Therefore, even samples of the same substance with small differences in impurity content (in the order of 0.1%) will have a visually recognizable thermograms (Figure 2).

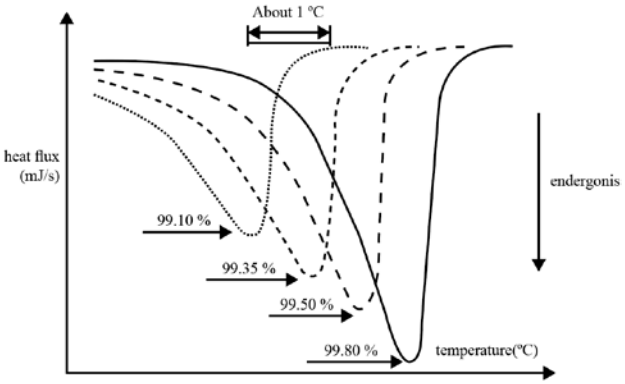


Figure 2. Thermograms depending on the purity

The determination of molar purity by DSC is based on the utilization of a mathematical approximation of the integrated form of the Van't Hoff equation applied to the concentrations (not activities) in a binary system.

$$[In(1 - x_2) \approx -x_2 \; and \; T \times T_0 \approx \; T_0^2]$$

If the impurity content x_2 is significantly less than 1 ($x_2 \ll 1$) and the temperature T is close to the melting point T_0, the equation can be written as follows, where T and x_2 are variables.

$$T = T_0 - (RT_0^2/\Delta H_f \times x_2)$$

T = Temperature of the sample in Kelvin

T_0 = Melting point of the chemically pure substance, in Kelvin

R = Ideal gas constant, in $J \cdot K^{-1} \cdot mol^{-1}$

ΔH_f = Molar heat of fusion of the pure substance, in $J \cdot mol^{-1}$

x_2 = Mole fraction of the impurity, which corresponds to the number of moles of the impurity divided by the total number of moles in the liquid phase (or molten phase) at temperature T (in Kelvin)

The determination of purity by DSC is limited to the detection of impurities that form a eutectic mixture with the principal compound and are present in molar fractions of less than 2% of the sample to be measured. This method is not applicable to the following:

– Amorphous substances

– Solvates or polymorphic compounds that are unstable within the experimental temperature range

– Impurities that form solid solutions with the principal substance

– Impurities that are insoluble in the liquid phase or in the melt of the principal substance

While heating the sample, the impurities are completely melted at the eutectic point. Above this temperature, the solid phase contains only the pure substance. As the temperature gradually increases from the eutectic point to the melting point of the pure substance, the amount of liquefied pure component increases, and therefore the mole fraction of impurities in the liquid phase decreases.

When the entire sample is melted above the eutectic point, F = 1 and $x_2 = x_{\hat{2}}$.

$$x_2 = 1/F \times x_{\hat{2}}$$

F = Molten fraction of the measured sample

$x_{\hat{2}}$ = Mole fraction of the impurity in the measured sample

Substituting equation (2) into equation (1) yields the following equation.

$$T = T_0 - (RT_0^2/\Delta H_f \times 1/F \times x_{\hat{2}})$$

The value of the heat of fusion of the pure substance is determined by the integration of the melting peak. The melting point T_0 of the pure substance is extrapolated from the plot of absolute temperature T (in Kelvin) vs. $1/F$. The slope α of the curve, straightened if necessary is equal to $RT_0^2\ x_{\hat{2}}/\Delta H_{\mathrm{f}}$,, which gives $x_{\hat{2}}$.

Multiply the mole fraction $x_{\hat{2}}$ by 100 to get the mole fraction (%) of total eutectic impurities.

Thin Layer Chromatography
박층크로마토그래프법

The Thin Layer Chromatography is a method to separate individual components by developing a mixture in a mobile phase on a thin layer of a suitable stationary phase and it is used for identification, purity test, etc. of substances.

Preparation of a thin layer plate

The plate for thin layer chromatography is usually prepared according to the following method.

Prepare a thin layer glass plate of 5 cm × 20 cm or 20 cm × 20 cm, coated with stationary phase (thickness: 0.2 or 0.25 mm). A suitable alumina or plastic plate may be used instead of the glass plate. The dried plates should be stored away from moisture.

Procedure

Unless otherwise specified, proceed by the following method.

Set the starting line at a height of about 20 mm from the bottom of the thin layer plate. Leave a margin of at least 10 mm on both sides. On the starting line, place the amount of the test solution and the standard solution specified in the monograph in a circular shape with a diameter of 2 to 6 mm and a distance of about 10 mm or more using a micropipette, etc., and air dry the plate. Then, unless otherwise specified, attach the filter paper along the inner wall of the chamber in advance and moisten the filter paper with the developing solvent. Pour the developing solvent again to a depth of about 10 mm, seal the chamber, and leave it at ordinary temperature for about 1 hour. When the tip of the developing solvent expands from the starting line to the distance specified in the monograph, remove the plate, immediately mark the end of the solvent, and dry it in the air. Compare the R_{f} value, color, etc. of each spot of the test solution and the standard solution according to the method specified in the monograph. Calculate the R_{f} value by using the following equation:

$$R_{\mathrm{f}} = \frac{\text{Distancd from the starting line to the center of the spot}}{\text{Distance from the starting line to the solvent front}}$$

Titrimetry
적정종말점검출법

The Titrimetry refers to the method used for volumetric analysis or its procedure. There are acid-base titration (neutralization titration or pH titration), precipitation titration, complexometric titration, and oxidation-reduction titration, depending on the type or phenomena of stoichiometric reaction that occurs between the titrated and the titrant solution (standard solution for volumetric analysis). In addition, titration in a non-aqueous solvent system is generally referred to as non-aqueous titration and is often used for the titration of weak acids, weak bases, or their salts. The endpoint of the reaction in titration can be identified by a change in the color of indicator or in the electrical signal (electric potential difference or current).

The indicator method is to determine the endpoint of titration by using the property that the color of the indicator dissolved in the titrated solution changes rapidly near the equivalence point. It is usually observed visually. Selection of an indicator and specification of the color change indicated the endpoint are specified in the monograph. Choose a suitable indicator which should be sensitive to slight changes in the (physical and chemical) property of liquid of the titrated solution, such as pH, near the equivalence point, and show changes in color.

The electrical endpoint detection methods include the potentiometric method and the amperometric method. The titration for them is referred to as the potentiometric titration and the amperometric titration, respectively, which are called as the electrometric titration collectively. In the potentiometric titration, the endpoint of titration is usually set as the point at which the change in electromotive force to titration amount is maximized. In the amperometric titration, unless otherwise specified, the constant-current polarization voltage (bi-amperometric) titration is used, where the endpoint is determined by the change in the minute current that changes as the titration progresses. Also there is a method of using coulometry (current × time) to track the changes in chemical reactions electrically, which is specified in the coulometric titration method for Water Determination.

In addition, the composition of the titration system [sample collection, dissolving solvent, standard solution for volumetric analysis, endpoint detection method, equivalent amount (mg) of titrated substance per mL of standard solution] is specified in the monograph. It is recommended that the standardization of the standard solution for volumetric analysis and the titration of the sample are performed under the same conditions, such as the measurement temperature, etc. If there is a significant difference in the temperature between 2 cases, the volume change of the standard solution should be corrected appropriately.

Method I. Indicator method

Put the sample of amount specified in the monograph or the

standard solution for volumetric analysis in an appropriate container, such as an Erlenmeyer flask, and dissolve it in a specified amount of solvent. Add the specified indicator to this solution and use it as the titrated solution. Titrate by dropping the standard solution for volumetric analysis from the burette. Carefully add a titrant solution of 0.1 mL or less before and after the endpoint and observe the change in the color of solution. Read the gradation on the burette for the amount of titration consumed from the start of titration until the change in the color specified in the monograph or standard solution for volumetric analysis is observed. Usually, the standard solution for volumetric analysis is dropped with a burette manually, but an automatic burette can also be used.

In the monograph or standard solutions for volumetric analysis, the phrase "perform a blank titration in a same way to make any necessary correction" usually means the following procedure:

Take the amount of a solvent specified in the monograph or standard solution for volumetric analysis and use it as the test solution. Calculate the amount of standard solution for volumetric analysis dropped until the specified color change occurs, which is used as the amount of the blank test solution. However, if the amount for the blank test is too small and cannot be determined accurately, it can be assumed to be 0 (mL).

Method II. Electrical endpoint detection methods

Potentiometric Titration Method

1) Apparatus

The apparatus consists of a beaker into which the sample is added, a burette from which the standard solution for volumetric analysis is dropped, an indicator electrode and a reference electrode, a potentiometer that measures the potential difference between 2 electrodes or a suitable pH meter, a recorder, and a stirrer that can gently stir the solution in the beaker. Also, an automatic titration apparatus that combines devices and parts or a data processing system, etc. required for the titration can be used.

In this test method, the indicator electrodes in the following table are used according to the type of titration, unless otherwise specified. In addition, a silver-silver chloride electrode is normally used as the reference electrode. However, a combined reference and indicator electrodes can also be used.

Type of titration	Indicator electrode
Acid-base titration (neutralization titration, pH titration)	Glass electrode
Precipitation titration (Titration of halide ions by silver nitrate)	Silver electrode. However, a silver-silver chloride electrode is used as a reference electrode. A salt bridge of saturated potassium nitrate solution is inserted between the reference electrode and the titrated solution.
Oxidation-reduction titration (Diazo titration etc.)	Platinum electrode
Complexometric titration	Mercury-mercury chloride (II) electrode
Non-aqueous titration (Perchloric acid titration, tetramethylammonium hydroxide titration)	Glass electrode

When the potentiometric titration is performed after measuring pH, the pH meter should be adjusted according to the pH Measurement.

2) Procedure

Put the sample of amount specified in the monograph in a beaker and dissolve it in the specified amount of the solvent. Wash the electrode well in advance with the solvent to be used, and place it in the solvent to be titrated. Stabilize the potential difference E (mV) or pH, and place the reference and indicator electrodes in the titration beaker containing the test solution. Titrate with the standard solution for volumetric analysis (titrant solution) while gently stirring the test solution. Immerse the tip of burette in the test solution, and measure the changes in potential difference whenever 0.1 mL or less of titrant solution is added dropwise before and after the endpoint carefully. Draw a titration curve by plotting the potential difference on the vertical axis and the dropped amount of *V* (mL) on the horizontal axis. Determine the maximum or minimum point of $\Delta E/\Delta V$, or electromotive force equivalent to the equivalence point, or dropped amount V representing pH, and set it as titration endpoint.

In addition, the blank test in the potentiometric titration is usually performed by the following procedure. Take the amount of the solvent specified in the monograph or standard solution for volumetric analysis, and use it as the test solution. Calculate the amount of dropped standard solution for volumetric analysis until the endpoint, which is used as the amount of the blank test solution. However, if the amount for the blank test is too small and cannot be determined accurately, it can be assumed to be 0 (mL)..

Unless otherwise specified, the titration endpoint is determined using one of the following methods.

A) Drawing method

On the titration curve obtained, draw two tangents parallel to each other with a gradient of about 45°. Draw another parallel line at the same distance from these 2 straight lines parallel to each other, and find out the point of intersection with the titration curve. From this point, draw the vertical line down to the horizontal axis to get the dropped amount, and set it as the endpoint of titration. Separately, draw a differential curve ($\Delta E/\Delta V$) and determine the endpoint of the titration based on the dropped amount, which represents the maximum or minimum.

B) Automatic detection method

When titrating with an automatic titrator, the endpoint can be determined automatically according to the instructions of each apparatus. The endpoint is determined as the point at which the rate of change of the potential difference reaches the maximum. Also, it can be determined as the dropped amount, where the indicated potential difference reaches the endpoint potential while the endpoint potential is set in advance.

Amperometric titration

1) Apparatus

The apparatus consists of a beaker into which a sample is placed, a burette from which the standard solution for volumetric analysis is dropped, two small platinum plates or platinum wires of the same shape as indicator electrodes, a voltage device to apply a very small DC voltage between two electrodes, a microammeter that measures the indicating current flowing between electrodes, a recorder, and a stirrer to gently stir the solution in the beaker. Also, an automatic titration apparatus that combines devices and parts or a data processing system, etc. required for the titration can be used.

2) Procedure

Put the sample of amount specified in the monograph, dissolve it in the specified amount of solvent. Place 2 indicator electrodes, which have previously been washed well with water, in the test solution. Then, apply a constant voltage suitable for the

measurement between the electrodes using an electrical voltage device, and titrate the test solution with the standard solution for volumetric analysis (titrant solution). Immerse the tip of burette in the test solution. Measure the change in current value whenever 0.1 mL or less of titrant solution is added dropwise before and after the endpoint carefully. Draw a titration curve by plotting the current value on the vertical axis and the dropped amount (mL) on the horizontal axis. The endpoint is usually determined as the dropped amount, which is the inflection point of the titration curve (point of intersection obtained by extrapolating the straight lines before and after the inflection point).

Unless otherwise specified, the titration endpoint is determined using one of the following methods:

i) Drawing method

In general, calculate the point of intersection by extrapolating the straight line before and after the inflection of the titration curve. The endpoint of the titration corresponds to an amount added dropwise indicated by this point.

ii) Automatic detection method

When titrating with an automatic titrator, the endpoint can be determined automatically according to the instructions of each instrument. The endpoint can be determined as the dropped amount, where the indicated potential difference reaches the endpoint potential while the endpoint potential is set in advance.

Regardless of whether the indicator method or the electrical endpoint detection method is used for the endpoint detection, if carbon dioxide, oxygen, etc., in the air may affect the results, use a sealed titration beaker and perform the test in an inert gas stream, such as nitrogen. If the results can be affected by light, avoid direct sunlight and use a light-resistant container.

Total Organic Carbon
유기체탄소시험법

The Total Organic Carbon (TOC) is a method for measuring the amount of carbon (organic carbon) that constitutes organic substances present in water. In general, organic carbon can be oxidized to carbon dioxide by dry decomposition, in which organic compounds are oxidized by combustion, or by wet decomposition, in which organic compounds are oxidized by applying ultraviolet rays or adding oxidizing agents. The amount of carbon dioxide produced in the decomposition process is measured using an appropriate method such as infrared gas analysis, electrical conductivity measurement, or resistivity measurement. From this value, the amount of organic carbon in the water is determined.

Carbon in water exists in two forms: organic and inorganic. When measuring the amount of organic carbon, the following two approaches can be applied. One method is to measure the total amount of carbon in the water and then subtract the amount of inorganic carbon from the total amount of carbon. The other method is to remove the amount of remaining organic carbon.

Apparatus

The apparatus consists of a sample injection port, a decomposition device, a carbon dioxide separation block, a detector, and a data processor or a recorder. The apparatus must measure organic carbon up to 0.05 mg or less per liter. The sample injection port has a structure that allows a sample to be injected with a micro syringe or a specific amount of the sample to be injected with a suitable sampling tool. The decomposition unit for the dry decomposition consists of a combustion tube and an electric furnace for heating, etc., which are controlled to a specified temperature according to the dry decomposition. In addition, the decomposition unit uses a device that can detect NLT 0.450 mg/L of carbon when measuring the amount of organic carbon in sodium dodecylbenzene sulfonate solution (0.806 mg/L). The carbon dioxide separation unit is a device that removes moisture in carbon dioxide produced by decomposition or a device that separates carbon dioxide from a decomposition product of the sample. The detector uses an infrared gas analyzer and an electrical conductivity meter, or resistivity meter to convert the carbon dioxide introduced from the carbon dioxide separator into an electrical signal proportional to its concentration. The data processing device calculates the organic carbon concentration in the sample from the electrical signal converted by the detector, and the recorder records the intensity of the electrical signal converted by the detector.

Reagents and standard solutions

Water used for measuring organic carbon (water for measurement) This is water used for preparing standard solutions or decomposition aids and the final washing of the test apparatus. When measuring the organic carbon in a container, use water with a carbon value of NMT 0.250 mg/L.

Standard potassium hydrogen phthalate solution Concentration determined as specified for each apparatus. After drying potassium hydrogen phthalate (standard reagent) at 105 ℃ for 4 hours and cooling it in a desiccator (silica gel), accurately weigh a certain amount and add water for measurement.

Standard solution for measuring inorganic carbon The concentration of this standard solution is determined as specified for each apparatus. Dry sodium bicarbonate in a desiccator (sulfuric acid) for at least 18 hours. Dry sodium carbonate (standard reagent) separately at 500 to 600 ℃ for 30 minutes then accurately weigh prescribed amounts with a carbon content ratio of 1:1, and dissolve in water for measurement.

Decomposition aid Dissolve a specified amount of potassium persulfate or a substance that can be used for the same purpose in water for measurement and adjust the concentration specified for each apparatus.

Gas for removing inorganic carbon or carrier gas Use nitrogen, oxygen, or other gases that can be used for the same purpose.

Acid for removing inorganic carbon Dilute hydrochloric acid, phosphoric acid, or other substances that can be used for the same purpose with water for measurement, and adjust the concentration as specified for the individual apparatus.

Apparatus

Sample container and reagent container Use materials that do not release organic carbon from the surface of the containers. Immerse the containers in a solution of diluted hydrogen peroxide (1 in 3) and a mixture of diluted nitric acid (1:1), and thoroughly rinse with measurement water.

Microsyringe Wash a microsyringe with a mixture of sodium hydroxide solution (1 in 20) and ethanol (99.5) (1 : 1) or dilute hydrochloric acid (1 in 4) and wash thoroughly with water for measurement.

Procedure

Employ an analytical method suitable for the apparatus used. Calibrate the apparatus using the standard potassium hydrogen phthalate solution with the procedure specified for the apparatus used. Ideally, integrate these devices into the water manufacturing line for testing. Otherwise, this test should be performed under clean conditions where the use of organic solvents

or other substances that could affect the test result is prohibited. Use a large sample container to collect a substantial volume of the water to be tested, and the measurement is conducted immediately after sample collection.

1) Measurement of organic carbon by subtracting inorganic carbon from the total amount of carbon

Follow the operational procedure of each apparatus. Introduce an appropriate sample size capable of accurately measuring the expected total carbon content into the sample injection port. Decompose organic and inorganic carbon in the sample, producing carbon dioxide detected by the detection unit. The total amount of carbon in the sample is measured by a data processing device or a recording device. Then, set up a device that measures only the amount of inorganic carbon in the sample and measures the amount of inorganic carbon by operating it in the same way as measuring the total amount of carbon. The amount of organic carbon can be obtained by subtracting the amount of inorganic carbon from the total carbon.

2) Measurement of the amount of organic carbon after removing inorganic carbon

Remove inorganic carbon by adding an acid for removing inorganic carbon to the sample and then injecting a gas for inorganic carbon (e.g., nitrogen) removal into the sample. According to the test procedure specified for the apparatus used, inject an appropriate amount of the sample with a measurable amount of organic carbon into the apparatus through the sample injection port and decompose the sample. Decompose the sample, detect the produced carbon dioxide, and calculate the organic carbon amount using a data processor or recorder. Also, for apparatus where the inorganic carbon is removed, first inject an appropriate amount of the sample with a measurable amount of organic carbon into the instrument through the sample injection port, according to the test procedure specified for the apparatus used. Remove the inorganic carbon by adding acid for inorganic carbon removal to the sample in the decomposition device and injecting the gas for inorganic carbon removal into the sample. Decompose the organic carbon, detect the carbon dioxide produced, and calculate the organic carbon amount using aa data processor or recorder.

Total Protein
총단백질정량법

Protein assay methods include the method that measures the physical properties of the protein itself, such as the ultraviolet absorption of amino acids constituting the protein, and the method that measures the absorbance after changing the absorption wavelength to within the range of visible light through the chemical reaction between a certain substance and protein. In the visible light absorption measurement, the chemical reaction develops color and the degree of such color is proportional to the protein amount, so it has the advantage of increasing sensitivity of the test since the absorbance is amplified.

Ultraviolet absorption spectrophotometry

Proteins in a solution absorb ultraviolet light at the wavelength of 280 nm mainly due to their structures with their aromatic amino acids, such as tyrosine and tryptophan. Using this property, proteins in solution can be quantified. If the buffer used to dissolve the protein has a relatively high absorbance relative to that of water, it indicates that an interfering substance is present in the buffer. This interference can be eliminated or minimized by using the same buffer as a calibration solution, but if the interfering substance produces high absorbance, the appropriate results cannot be obtained. If the protein concentration to be measured is low, the measured value may significantly decrease as the protein is adsorbed onto the cell. This can be resolved by increasing the protein concentration or by using non-ionic surfactants when preparing buffer solution.

Procedure

During measurement, keep the test and standard solution, standard solution and buffer solution at the same temperature. Use the prepared buffer solution as the calibration solution. Put the test and standard solutions into a quartz cell, and measure the absorbance at 280 nm. The value of the protein concentration can be calculated correctly when the corresponding absorbance falls within the range of the calibration curve, which is a straight line.

1) Test solution

Dilute the sample to be measured with the prepared buffer to make the protein concentration between 0.2 and 2 mg/mL.

2) Standard solution

Dilute the reference standard to be measured with the same buffer used in preparing the test solution to have the same protein concentration range as in the test solution.

Light scattering

The accuracy of UV spectroscopy of proteins can be reduced when there is light scattering by the sample. If the proteins in a solution have a size similar to the wavelength used for analysis (250 nm to 300 nm), the absorbance of the sample will definitely increase due to light scattering. To calculate the absorbance by light scattering at 280 nm, measure the absorbance of the test and standard solution at wavelengths of 320 nm, 325 nm, 330 nm, 335 nm, 340 nm, 345 nm and 350 nm, and draw a graph with the logarithmic value of these measured values and the logarithmic value of each measured wavelength. Next, use linear regression to create a standard curve that accurately represents the coordinate values on the graph. After calculating the logarithmic value of the absorbance at 280 nm by extrapolation of this curve, the antilogarithm value is calculated, then it is the absorbance by light scattering at 280 nm. Total absorbance measured at 280 nm minus the absorbance attributed to light scattering is the absorbance of protein in the solution Filtration with a 0.2 μm filter that does not adsorb protein or clarification by centrifugation may be performed to reduce the effect of light scattering, especially if the solution is noticeably turbid.

Calculations

The protein concentration in the test and standard solution is calculated using the correction value in the following formula. If necessary, the extinction coefficient can be applied.

$$C_U = C_S(A_U/A_S)$$

C_U: Protein concentration in the test solution
C_S: Protein concentration in the standard solution
A_U: Corrected absorbance value of the test solution
A_S: Corrected absorbance value of the standard solution

Lowry method

The Lowry method is based on the principle that the chromogen of mixed acid of phosphomolybdic acid/phosphotungstic acid in Folin TS (phosphomolybdic acid/phosphotungstic acid TS or Folin-Ciocalteu phenol TS) is reduced by protein, and the color developed shows the maximum absorbance at a wavelength of 750 nm. Folin TS (phosphomolybdic acid/phosphotungstic acid TS) reacts mainly with tyrosine residues in proteins, and

color development reaches a maximum at 20 – 30 minutes at ordinary temperature, and then the color gradually disappears. As this method is sensitive to interfering substances, it is employed following the precipitation of proteins from the sample. Most interfering substances decrease color development, but some surfactants slightly increase color development. A high salt concentration can precipitate the conjugates of phosphomolybdic acid/phosphotungstic acid and protein. As a color development reaction of different intensities can be exhibited depending on the type of protein, the reference standard and samples use the same type of protein, when possible. If there is no reference standard to fit, replace it with albumin for protein quantification. To isolate the interfering substances from the proteins in the sample, follow the method for removal of the interfering substances described below before preparing the test and standard solution. If the concentration of the sample is sufficiently high, the effect of the interfering substances can be minimized by increasing the dilution factor for accurate measurement.

Procedure Add 1 mL of alkaline copper TS each to 1 mL of the standard solution, the test and standard solution, and the blank test solution, and mix by shaking. After allowing to stand for 10 minutes, add 0.5 mL of Folin TS (phosphomolybdic acid/phosphotungstic acid TS) diluent, and mix. Allow to stand at ordinary temperature for 30 minutes. Measure the absorbance at 750 nm, using the blank test solution as a calibration solution.

1) Test solution Dilute the proper amount of sample with the prepared buffer to make a test solution that falls within the range of the standard curve. The optimum pH range of the buffer is 10.0 to 10.5.

2) Standard solution Dissolve the reference substance for the protein to be determined in the prescribed buffer. Dilute it with the same buffer to obtain standard solutions of not fewer than five different concentrations evenly spaced over a suitable range situated between 5 ug/mL and 100 ug/mL.

3) Blank test solution Use the buffer used to prepare the test and standard solutions.

4) Copper sulfate TS Dissolve 100 mg of copper sulfate and 0.2 g of sodium tartrate in water to make 50 mL. Dissolve 10 g of anhydrous sodium carbonate in water to make 50 mL. Slowly add sodium carbonate solution to copper sulfate solution and mix. Use within 24 hours.

5) Alkaline copper TS Mix 1 volume of copper sulfate TS, 2 volumes of sodium dodecyl sulfate solution (50 g/L) and 1 volume of sodium hydroxide solution (32 g/L). Store at ordinary temperature and use within 2 weeks.

6) Folin TS (Phosphomolybdic acid/phosphotungstic acid TS) Dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700 mL of water, then add 100 mL of hydrochloric acid and 50 mL of phosphoric acid. Heat for 10 hours with a reflux condenser. Add 150 g of lithium sulfate, 50 mL of water and a few drops of bromine, and boil for about 15 minutes to remove excess bromine. After cooling down, add water to make it up to 1000 mL and filter. The color of this solution should be yellow. If it is green in color, the solution is not suitable. In this case, it can be prepared again by adding a few drops of bromine and boiling again.

7) Folin TS (Phosphomolybdic acid/phosphotungstic acid TS) diluent Mix 5 mL of Folin-ciocalteu TS with 55 mL of water. Store in an amber bottle at ordinary temperature.

8) Deoxycholic acid-trichloroacetic acid Mixture of sodium deoxycholate and trichloroacetic acid (1 : 1)

Sodium deoxycholate

Dissolve 150 mg of sodium deoxycholate in 100 mL of water to make 1.5 g/L.

Trichloroacetic Acid.

Dissolve 72 g of trichloroacetic acid in 100 mL of water to make 720 g/L.

Interfering substance To remove interfering substances, deoxycholic acid-trichloroacetic acid is added to the sample to precipitate proteins before measurement. This method can also be used for concentrating proteins from a dilute solution.

Add 0.1 mL of a 1.5 g/L solution of sodium deoxycholate reagent to 1 mL of a solution to be examined. Mix using a vortex mixer and allow to stand at ordinary temperature for 10 min. Add 0.1 mL of a 720 g/L solution of trichloroacetic acid reagent and mix using a vortex mixer. Centrifuge at 3000 g for 30 min, decant the liquid and remove any residual liquid with a pipette. Redissolve the protein pellet in 1 mL of alkaline copper TS.

Calculations The relationship of absorbance to protein concentration is non-linear; however, if the range of concentrations used to prepare the standard curve is sufficiently small, the latter will approach linearity. Plot the absorbances of the standard solutions against the protein concentrations and use linear regression to establish the standard curve. From the standard curve and the absorbance of the test and standard solution, determine the concentration of protein in the test solution.

Bradford protein assay

This method works on the principle that the maximum absorption wavelength shifts from 470 nm to 595 nm when acid blue 90 dye binds to protein.

Acid blue 90 dye binds most readily to arginine and lysine residues in the protein which can lead to variation in the response of the assay to different proteins. Therefore, the protein content of the sample should be measured using the same type of protein as a reference standard. There are few interfering substances, but it is preferable to avoid surfactants and amphoteric electrolytes in the sample. Strongly alkaline samples may interfere with the reaction of acidic solution TS.

Procedure Add 5 mL of acid blue 90 TS to 0.1 mL each of standard solution, test and standard solution, and blank test solution, and mix by inversion. Make sure not to make any bubbles at this time, as the formation of bubbles can reduce reproducibility. Measure the absorbance of the standard and the test and standard solutions at 595 nm with the blank test solution as a calibration solution. Do not use a quartz cell as the dyes may bind to this material.

1) Test solution Dilute the proper amount of sample with the prepared buffer to make a test solution that falls within the range of the standard curve.

2) Standard solution Dissolve the reference substance in the prepared buffer. Make standard solutions of at least 5 different concentrations to ensure that the concentrations are evenly distributed within range of 0.1 – 1 mg/mL of protein.

3) Blank test solution Use the buffer which is used to prepare the test and standard solutions.

4) Acid blue 90 TS Dissolve 0.1 g of acid blue 90 in 50 mL of alcohol. After adding 100 mL of phosphoric acid to this solution, dilute it with water to make it up to 1000 mL and mix. Filter and store in an amber bottle at ordinary temperature. As the dyes slowly precipitate when stored, it should be filtered before use.

Calculations The relationship of absorbance to protein concentration is non-linear; however, if the range of concentrations used to prepare the standard curve is sufficiently small, the latter will approach linearity. Plot the absorbances of the standard solutions against the protein concentrations and use linear regression to establish the standard curve. From the standard

curve and the absorbance of the test and standard solution, determine the concentration of protein in the test solution.

Bicinchoninic acid (BCA) assay

The bicinchoninic acid method uses a reaction in which divalent copper ions (Cu^{2+}) are reduced to monovalent copper ions (Cu^{1+}) by proteins. Bicinchoninic acid TS is used for providing monovalent copper ions (Cu^{1+}). Few substances interfere with the reaction. But if there is an interfering substance in the sample, its effect can be minimized by dilution. However, a sufficient amount of the sample must be present to accurately measure its protein concentration. Alternatively, interfering substances can be removed by precipitating proteins from the sample, as in the Lowry method. Since different types of protein can show different levels of color development, the reference standard protein and the sample protein must be the same.

Procedure Add 2 mL of copper-bicinchoninic acid TS to 0.1 mL each of the standard solution, test and standard solution, and the blank. Mix and incubate at 37 °C for 30 minutes. Record the time and let it cool down to ordinary temperature.

The reaction solution is transferred to a quartz cell within 60 minutes, and the absorbance is measured at 562 nm using the blank test solution as a calibration solution. After the solutions have cooled to ordinary temperature, the color intensity continues to increase gradually.

1) Test solution Dilute the proper amount of sample with the prepared buffer to be within the range of the concentrations of the standard solutions.

2) Standard solution Dissolve the reference substance in the prepared buffer. Make standard solutions of at least 5 different concentrations to ensure that the concentrations are evenly distributed within range of10 – 1200 μg/mL of protein.

3) Blank test solution Use the buffer which is used to prepare the test and standard solutions.

4) Bicinchoninic acid TS (BCA TS) Dissolve 10 g of disodium bicinchoninate, 20 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate, 4 g of sodium hydroxide, and 9.5 g of sodium hydrogen carbonate in water. Add sodium hydroxide solution or sodium hydrogen carbonate solution if necessary to adjust its pH to 11.25, and dilute with water to make it up to 1 L.

5) Copper-bicinchoninic acid TS Mix 1 mL of copper sulfate solution (4 g/L) and 50 mL of bicinchoninic acid TS.

Calculations The relationship of absorbance to protein concentration is non-linear; however, if the range of concentrations used to prepare the standard curve is sufficiently small, the latter will approach linearity. Plot the absorbances of the standard solutions against the protein concentrations and use linear regression to establish the standard curve. From the standard curve and the absorbance of the test and standard solution, determine the concentration of protein in the test solution.

Biuret method

In this method, when divalent copper ions (Cu^{2+}) are reacted with a protein solution under alkaline conditions, the color developed is measured at absorbance at 545 nm. Unlike other methods, this method shows a very small difference in the level of color development, although the protein types are different. For example, this test shows minimal difference between the same amount of immunoglobulin-G and albumin samples. However, when the sodium hydroxide solution is added to biuret TS, immunoglobulin-G may exhibit a higher absorbance than albumin if it is not mixed sufficiently or if a prolonged reaction time passes after mixing. The trichloroacetic acid method used to minimize the effects of interfering substances also can be used to determine the protein content in the test and standard solution at concentrations below 500 μg/mL.

Procedure To one volume of the test and standard solution, add an equal volume of sodium hydroxide solution (60 g/L) and mix. Immediately add biuret TS equivalent to 0.4 volumes of the test and standard solution, and mix rapidly. Incubate for at least 15 minutes at 15 - 25 ℃. Measure the maximum absorbance at 545 nm within 90 minutes after adding biuret TS using the blank test solution as a calibration solution. Any solution that develops turbidity or a precipitate is not acceptable for use.

1) Test solution Dilute the proper amount of sample with sodium chloride solution (9 g/L) to make a sample solution which falls within the concentration range of the standard solution.

2) Standard solution Dissolve reference standard substance in sodium chloride solution (9 g/L) to make standard solutions of at least 3 different concentrations to ensure that the concentrations are evenly distributed within the concentration range of 0.5 - 10 mg/mL of protein.

3) Blank test solution Sodium chloride solution (9 g/L) is used.

4) Biuret TS Dissolve 3.46 g of copper sulfate in hot water to make 10 mL and allow to cool (solution A). Dissolve 34.9 g of sodium citrate (dihydrate) and 20.0 g of sodium carbonate anhydrous in 80 mL hot water and allow to cool (solution B). After mixing Solution A and Solution B, dilute with water to make 200 mL. Use this mixture within 6 months. Do not use the reagent if it develops turbidity or contains any precipitate.

Calculations The relationship of absorbance to protein concentration is approximately linear within the indicated range of protein concentrations for the standard solutions. Plot the absorbances of the standard solutions against protein concentrations, and use linear regression to establish the standard curve. Calculate the correlation coefficient for the standard curve. A suitable system is one that yields a line having a correlation coefficient of NLT 0.99. From the standard curve and the absorbance of the test and standard solution, determine the concentration of protein in the test and standard solution.

Interfering substance

To minimize the effects of interfering substance, proteins in the sample can be precipitated and used as follows. Add 0.1 volumes of trichloroacetic acid solution (500 g/L) to 1 volume of a test solution centrifuge. Discard the supernatant and dissolve the precipitate in a small amount of sodium hydroxide solution (0.5 mol/L).

Turbidity
탁도시험법

The Turbidity is used to determine the turbidity for the decision whether the article to be examined complies with the clarity requirement stated in the Purity.

As a rule, the visual method is specified for the requirement in individual monograph.

Visual method

This method is used to determine the turbidity with white (or faintly-colored) fine particles. So the turbidity of a colored sample often recognized less distinctly, making accurate comparison challenging without the use of similarly colored reference suspension.

Turbidity control solution Pipet 5 mL, 10 mL, 30 mL

and 50 mL of formazine standard suspension, dilute them separately to exactly 100 mL with water, and use these solutions so obtained as Reference suspensions I, II, III and IV, respectively. Shake before use. Turbidity of Reference suspensions I, II, III and IV are equivalent to 3 NTU, 6 NTU, 18 NTU and 30 NTU, respectively.

Procedure Place sufficient of the test solution, water or the solvent to prepare the test solution and, where necessary, newly prepared Reference suspensions in separate flat-bottomed test tubes. The tubes should have an internal diameter of 15 – 25 mm, be colorless, transparent, and filled to a depth of 40 mm. Compare the contents against a black background in diffused light down the vertical axes of the tubes. The diffused light must be such that Reference suspension I can be readily distinguished from water, and that Reference suspension II can readily be distinguished from Reference suspension I.

In this test Reference suspensions are used when the clarity of the test solution is obscurely and it is not easy to determine that its turbidity is similar or not similar to water or to the solvent used to prepare the test solution.

Interpretation A liquid is considered "clear" when its clarity is the same as that of water or of the solvent used to prepare the liquid or its turbidity is not more pronounced than that of Reference suspension I. If the turbidity of the liquid is more than that of Reference suspension I, consider as follows: When the turbidity is more than that of Reference suspension I but not more than that of Reference suspension II, express "it is not more than Reference suspension II". Similarly, when the turbidity is more than that of Reference suspension II but not more than that of Reference suspension III, express "it is not more than Reference suspension III", and when the turbidity is more than that of Reference suspension III but not more than that of Reference suspension IV, express "it is not more than Reference suspension IV". If the turbidity exceeds Reference Suspension IV, express it as "more than Reference Suspension IV."

Test solutions Formazine standard suspension: To exactly 3 mL of formazine standard suspension add water to make exactly 200 mL. Use within 24 hours after preparation. Shake thoroughly before use. Degrees of opalescence of this standard solution is equivalent to 60 NTU.

Photoelectric photometry

The turbidity can also be estimated by instrumental measurement of the light absorbed or scattered on account of submicroscopic optical density in homogeneities of opalescent solutions and suspensions Photoelectric Photometry offers a more objective determination than the visual method. Though they can determine the turbidity by measuring the scattered or transmitted light, the measuring system and light source must be specified in individual test method, and for the comparison of observed data, the same measuring system and light source should be used.

In each case, the linear relationship between turbidity and concentration must be demonstrated by constructing a calibration curve using at least 4 concentrations. For colored samples, the turbidity value is liable to be estimated lower because of attenuating both incident and scattered lights due to the absorption by the color, and the transmission-dispersion method is principally used.

Turbidimetry When a light passes through a turbid liquid the transmitted light is decreased by scattering with the particles dispersed in the liquid. A linear relationship is observed between turbidity and concentration when the particles of constant size are uniformly dispersed, the size is small, and the suspension is not of high concentration. The turbidity can be measured by the Ultraviolet-visual Spectrophotometry using spectrophotometer or photoelectric photometer. The turbidity of the samples with high concentration can also be measured, however, it is susceptible to the color of the sample. Usually, measurements are performed around 660 nm to avoid possible disturbance from color absorption.

Nephelometry When a suspension is viewed at right angles to the direction of the incident light, it appears opalescent due to the refraction of light from the particles of the suspension (Tyndall effect). A certain portion of the light entering a turbid liquid is transmitted, another portion is absorbed and the remaining portion is scattered by the suspended particles.

The scattered light measuring method shows the linear relationship between the nephelometric turbidity units (NTU) values and relative detector signals in a low turbidity range. As turbidity increases, not all the particles are exposed to the incident light and the scattered light of other particles is hindered on its way to the detector.

Ratio turbidimetry This method measures both scattered and transmitted light values at the same time, and the turbidity is determined from the ratio of the scattered light value to the transmitted light value. This procedure compensates for the light that is diminished by the color of the sample and eliminates the influence of the color. When using an integrating sphere, particularly called the integrating sphere method, the total transmitted light value and scattered light value caused by suspended particles are measured, and the turbidity is determined from their ratio.

Application of Photoelectric Photometry for monograph requirements The turbidity of the test solution, determined by the photoelectric photometry, can be used as an indicating standard for the conformity to the clarity requirements by converting into NTU by using turbidity known reference solutions such as Reference suspensions I – IV, if needed, and water or the solvent used. In an automatically compensable apparatus calibrated with turbidity reference solutions, the measuring result is given in NTU, directly comparable with the required specified value.

NTU is often used as the unit in the turbidity determinations. It is the unit used when the turbidity is estimated by the instrument which measures the 90 ± 30° scattered light against the incident light intensity, using tungsten lamp. When the estimation is performed by the instrument which measures the 90 ± 2.5° scattered light against the incident light intensity using 860 nm infrared light, FNU is used as the unit. FNU is equivalent to NTU at a range of smaller measurements (less than 40 NTU). For the unit of formazin concentration, FTU (formazine turbidity units) is also used, which is defined as a suspension of 1 mg formazin in 1L of purified water is 1 FTU.

Ultraviolet-visible Spectroscopy
자외가시부흡광도측정법

The Ultraviolet-visible Spectroscopy is a method to perform the identification, purity test and assay of a substance by measuring the degree of absorption of light by the substance in a wavelength range between 200 and 800 nm. However, when an atomic absorption spectrometer is used, proceed as directed under the Atomic Absorption Spectrophotometry.

When monochromatic light passes through a substance in solution, the ratio of the transmitted intensity, P, to incident light intensity, P_0, is referred to as transmittance, t. Transmittance expressed in percentage is called percent transmittance, T. The

common logarithm of the reciprocal of transmittance is called absorbance, *A*.

$$t = \frac{P}{P_0}$$

$$T = \frac{P}{P_0} \times 100 = t \times 100$$

$$A = log\frac{P_0}{P}$$

The absorbance, *A*, is proportional to the concentration, *c* of a substance in the solution and the path length, *l*.

$$A = k \times c \times l$$ (*k* is the absorption coefficient.)

The absorbance, calculated when *l* is 1 cm and *c* is 1 mol/L, is called the molar absorption coefficient ε. The molar absorption coefficient at the wavelength of maximum absorption is expressed as ε_{max}.

When a light passes through a solution of a substance, the absorbance of a sample varies with the wavelength of the light. Therefore, the ultraviolet-visible absorption spectrum (hereinafter referred to as absorption spectrum) can be obtained by measuring the absorbance values of light at slightly different wavelengths, and then drawing a curve representing the relationship between absorbance and wavelength. From this absorption spectrum, the wavelengths of maximum absorption (λ_{max}) and wavelength of minimum absorption (λ_{min}) can be determined. Also, as the absorption spectrum is determined by the chemical structure of a substance, the substance can be identified by measuring its absorption spectrum within a specific wavelength range and comparing it with the absorption spectra of the reference standards. This can be done by determining the wavelength of maximum absorption, or by determining the ratio of absorbance at two specific wavelengths. Moreover, substances can be tested for identification or purity determination by measuring the wavelengths of maximum or minimum absorption or by determining the ratio of absorbance at two specific wavelengths. The concentration of a substance can be quantified by measuring the absorbance of a solution of a certain concentration at the wavelength of maximum absorption and comparing it with the absorbance of a standard solution of a certain concentration.

Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for measuring absorbance. Before measurement, adjust the spectrophotometer or photoelectric photometer based on the operation manual that came with the apparatus to confirm that the wavelength and the transmittance meet the specifications of the test method described below.

For wavelength calibration, use optical filters and measure transmittance at the wavelength near the standard value indicated in the test report. The deviation between the measured and standard wavelengths should be within ± 0.5 nm, and when the measurement is repeated three times, each measurement should be within ± 0.2 nm from the average value. The test can also be performed using a low-pressure mercury lamp at the bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm, and 546.07 nm, or a deuterium discharge lamp at the bright line wavelengths of 486.00 nm and 656.10 nm. At this time, the difference between the measured wavelength and the wavelength of the bright line should be within ± 0.3 nm, with each measurement within ± 0.2 nm of the average when repeated three times.

As for transmittance or absorbance, when a test is performed to determine the transmittance at the reference wavelengths indicated in the test report using the optical filters for calibrating wavelength under the test conditions provided in the test report that came with each filter, the difference between the measured transmittance and the reference transmittance should be between 1% larger than the upper limit of the relative precision indicated in the test report and 1% smaller than the lower limit of the relative precision, and when the measurement is repeated three times, each measurement of absorbance (or each value obtained by converting the measurement of transmittance into absorbance) should be within ± 0.002 from the average value, if the absorbance is equal to or smaller than 0.500, and within ± 0.004 from the average value, if the absorbance is larger than 0.500. It is also recommended to verify the linearity of transmittance by using multiple optical filters for calibrating wavelength which show different transmittances at the same wavelength.

Procedure

Before measurement, adjust the apparatus as directed in the "Apparatus and adjustment" section, and then select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width, scanning speed, etc. Start the apparatus and leave it for a certain time to see if it operates stably Close the shutter of the test solution's light path, block the light, and adjust transmittance to 0% at the measuring wavelength or range. Then, open the shutter again and adjust the indicated value of transmittance at the measuring wavelength or wavelength range to 100% (or adjust the absorbance to 0). Place the cell containing the control solution into the light path. Usually after placing the cell containing the control solution into the light path for the sample or for the control, adjust the indicated value of transmittance to 100 % (or adjust the absorbance to 0). Unless otherwise specified, use the blank solvent used in the test as the control solution. Next, place the cell containing the solution to be measured into the light path for the sample and measure the absorbance at the measuring wavelength or acquire the absorption spectrum at the measuring wavelength range. Unless otherwise specified, use a cell with a path length of 1 cm, which is made of quartz for the ultraviolet range and of quartz or glass for the visible range, for the measurement of absorbance. Pay special attention to solvent absorption in the ultraviolet range and use a solvent that does not interfere with accurate measurement.

Specific absorbance

The absorbance of a substance, of which concentration, *c*, is converted to a 1 w/v% solution and which is measured via spectroscopy over a 1-cm layer length (*l*), is called the specific absorbance, which is expressed as $E_{1cm}^{1\%}$.

$$E_{1cm}^{1\%} = \frac{A}{c \times l}$$

l: Path length of the solution layer (cm)
A: Absorbance
c: Concentration of the solution (w/v%)

For example, in the description in the monograph , $E_{1cm}^{1\%}$ (241 nm): 500 to 530 (after drying, 2 mg, methanol, 200 mL) indicates that $E_{1cm}^{1\%}$ of the substance is between 500 and 530 in the test, in which the sample is dried under the conditions specified in the Loss on drying' section for the substance, and then about 2 mg of the sample is weighed accurately with a microbalance and dissolved in methanol to make exactly 200 mL, then the

absorbance of the solution is measured at a wavelength of 241 nm over a layer length of 1 cm.

Identification

Prepare the test solution according to the specifications in the monograph, and test it as directed in the procedure. Usually, the test is carried out using a single method or in a combination of the following methods using the absorbance or absorption spectrum obtained from the test solution. However, minor differences in the absorption spectrum, assumed to be caused by variations in the apparatus, may be disregarded.

1) Identification using the reference standards When the absorption spectrum obtained from the test solution exhibits similar intensities of absorption at the same wavelengths as that obtained from a reference standard, the sample can be identified as the same substance as the reference standard.

2) Identification using the absorption wavelength When the wavelength of maximum absorption of an absorption spectrum obtained from the test solution matches the wavelength range specified in the monograph, the sample can be confirmed to be the same substance as described in the monograph.

3) Identification using the ratios of absorbance Determine the absorbance ratios at two or more wavelengths in the absorption spectrum of the test solution. Compare these ratios with those specified in the monograph. If they align with the monograph specifications, confirm that the sample is the same substance as described in the monograph.

Assay

Prepare the control solution, the test solution, and the standard solutions as directed in the monograph, measure the absorbance of the test solution and the standard solutions according to the method described in the procedure, and compare their absorbance values to quantify the concentration of the substance in the sample. Alternatively, assay can be conducted by measuring the absorbance values of the test solution and the standard solutions and applying them to an equation specified in the monograph.

Uniformity of Dosage Units
제제균일성시험법

To ensure the consistency of dosage units, each dosage unit in a batch should have an active ingredient content within a close range around the labeled amount. Dosage units are defined as dosage forms containing a single dose or a part of a dose of the active ingredient in each unit. However, this test is not applied to preparations intended for external cutaneous administration for local actions in the form of solutions, suspensions, emulsions, or gels contained in single-unit containers.

"Uniformity of dosage units" is defined as the degree of uniformity in the content of the active ingredient among dosage units. Therefore, the requirements of the Uniformity of Dosage Units apply to each active ingredient comprised in dosage units containing one or more active ingredients, unless otherwise specified.

The uniformity of dosage units is tested by either of the two methods listed in Table 1, Content Uniformity Test or Mass Variation Test. The content uniformity test is to determine whether the content of each active ingredient is within the allowable range by measuring the content of each active ingredient in each dosage unit, and the test can be applied in all cases of preparations. The mass variation test is a test where variations in the mass of individual dosage units are regarded as variations in the content, and the content uniformity of the active ingredient in the dosage unit is estimated by measuring the mass of each dosage unit

Table 1. Application of the content uniformity and mass variation tests for each preparation

Dosage form	Type	Sub-type	Content/Concentration of active ingredient	
			≥ 25 mg & ≥ 25%	< 25 mg & < 25%
Tablets	Uncoated	-	MV	CU
	Coated	Film-coated	MV	CU
		Other preparations	CU	CU
Capsules	Hard	-	MV	CU
	Soft	Suspensions, emulsions, and gels	CU	CU
		Solutions	MV	MV
Solid preparations in single-dose packages	Single component		MV	MV
	Multiple components	Solutions freeze-dried in final containers	MV	MV
		Other preparations	CU	CU
Dissolved liquid preparations in single unit containers			MV	MV
Other dosage forms			CU	CU

CU: Content Uniformity Test
MV: Mass Variation Test
* Dosage forms not addressed by the other categories in this table, including suppositories. transdermal systems (patches) and semi-solid preparations applied cutaneously and intended for systemic distribution of the drug substance.

The mass variation test is applied to the following:

(1) Preparations in which all ingredients are completely dissolved and homogenized to form a solution in single-unit containers (including soft capsules).

(2) Preparations containing only one component without any drug substance and additives, solids, such as powders, granules, and injections to be dissolved before use, packaged in single-dose containers.

(3) Solid preparations such as sterile injectable powders packaged in single-dose containers, prepared by completely dissolving all ingredients such as the drug substance or additives and free-drying in the final containers, and for which the method of preparation is indicated in the label or inserted sheet.

(4) Hard capsules, uncoated tablets, and film-coated tablets containing ≥ 25 mg of an active ingredient, and in which the active ingredient comprises ≥ 25% of the dosage unit by mass; however, coating layers and empty capsules that do not contain the active ingredient are not included in the calculation and if there are two or more active ingredients, each ingredient is calculated separately.

(5) The applicable active ingredient of a preparation in which the content of that ingredient deviates from the allowed variation of 10% of the labeled amount.

Preparations that do not satisfy the above conditions are subject to the content uniformity test. However, even when the 25mg/25% threshold limit is not met for a preparation specified in (4) above, if the data obtained from the validation on the manufacturing process and the formulation development show that the relative standard deviation (RSD) of the active ingredient's

concentration in the final dosage unit is ≤ 2% and a change in the test to the mass variation test has been approved, the mass variation test can be applied. The RSD of the active ingredient's concentration is the relative standard deviation of the active ingredient's concentration (w/w, w/v) for each dosage unit, and the active ingredient's concentration is obtained by dividing the active ingredient's content in each dosage unit by the mass of each dosage unit. Table 2 shows the general equation used for calculating the RSD.

1. Content uniformity test

Take NLT 30 samples and proceed with the test as follows. Where different methods are used for the assay of the preparations and the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

Solid preparations

Take 10 samples, determine the content of the active ingredient in each sample using an appropriate method, and calculate the acceptance value, referring to Table 2.

Liquid preparations or semi-solid preparations

Take 10 samples, thoroughly mix the content of each container according to the general use instructions, take the content from each container, determine the content of the active ingredient using an appropriate analytical procedure and present the result values with estimated content values to calculate the acceptance value, referring to Table 2.

Calculation of Acceptance Value

Calculate the acceptance value using the equation below:

$$|M - \overline{X}| + ks$$

the terms of which are defined in Table 2.

2. Mass variation test

This test is performed based on the assumption that the concentration of the drug substance (a value obtained by dividing the mass of the drug substance by the mass of the dosage unit) is uniform.

Obtain the average content of the drug substance by measuring samples that are representative of a batch using an appropriate analytical procedure. When this value is used as A, it is expressed as a% of the labeled amount as described in the section of Calculation of Acceptance Value. Take at least 30 samples and proceed with the test as follows.

1) Uncoated or film-coated tablets

Take 10 tablets as the samples, weigh the mass of each tablet accurately, and calculate the average content according to the assay. Obtain the estimated content value of each tablet based on this and express it as a% of the labeled amount, and then calculate the acceptance value.

2) Hard Capsules

Take 10 capsules as the samples, weigh each capsule accurately while taking care to match each capsule with the corresponding mass. Remove the content from each capsule using an appropriate method and accurately weigh the mass of each empty shell. Subtract the mass of each empty shell from the mass of the matching capsule to obtain the mass of the content individually. Calculate the average content of each capsule using the mass of the content and the average content obtained according to the assay, express it as a% of the labeled amount, and then calculate the acceptance value.

3) Soft capsules

Take 10 capsules as the samples, weigh each capsule accurately including the capsule shell while taking care to match each capsule with the corresponding mass. Then, cut the capsules open and wash out the content using a suitable solvent. Leave the capsules at ordinary temperature for about 30 minutes to allow the remaining solvent to be removed by evaporation. At this time, it is necessary to prevent the capsules from absorbing moisture or drying out. Weigh the mass of each empty shell accurately, and subtract the mass of each empty shell from the mass of the matching capsule to obtain the mass of the content individually. Calculate the average content of each capsule using the mass of the content and the average content obtained according to the assay, express it as a% of the labeled amount, and then calculate the acceptance value.

4) Solid preparations other than tablets and capsules

Proceed as directed for Hard capsules, treating each dosage unit as described therein. Calculate the acceptance value.

5) Liquid preparations

Take 10 samples and accurately weigh the mass of the oral liquids as directed under general use instructions. If necessary, measure the density and convert the value to the corresponding volume. Calculate the average content of each oral liquid sample using the mass or volume of the liquid and the content obtained according to the assay, express it as a% of the labeled amount, and then calculate the acceptance value.

2.1. Calculation of Acceptance Value

Calculate the acceptance value as described in the section of 'Content Uniformity Test' above. However, when $\overline{X}$ is A, replace the content of the drug substance in each sample with the estimated content of the drug substance as indicated below.

x_1, x_2, …, x_n: The estimated value of the drug substance in each sample tested.

$$x_i = w_i \times \frac{A}{\overline{W}}$$

w_1, w_2, …, w_n: The mass of each sample tested.

A: The content of the drug substance (% of the labeled amount) obtained by measurement using an appropriate method.

$\overline{W}$: The average mass of each sample tested (w_1, w_2, …, w_n) .

3. Interpretation criteria

Apply the following criteria, unless otherwise specified.

1) Solid preparations, semi-solid preparations, and liquid preparations

When the acceptance value is calculated using the first 10 samples, if the value is less than or equal to $L1$%, such dosage form is considered to meet the requirements. If the acceptance value exceeds $L1$%, take another 20 samples, test them in the same way, and calculate the acceptance value again. When the acceptance value is calculated from 30 samples used for two tests, if the value is less than or equal to $L1$% and at the same time no individual content of the dosage unit is less than (1- $L2$ × 0.01) M nor more than (1- $L2$ × 0.01) M as indicated in the section "Calculation of Acceptance Value" for the content uniformity

test or the mass variation test, such dosage form is considered to meet the requirements. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

2) For cases subject to (5) of preparations to which mass variation is applied.

A) Granules, powders, syrups, and liquid preparations (distribution)

When the average mass is calculated by accurately weighing 20 samples, if the variation between the average value and the mass of each sample is ≤ 10%, such preparation is considered to meet the requirements. When there is any sample for which mass deviates >10% from the average, proceed to the following test with the content. Take 20 samples and weigh the mass of each sample accurately. At this time, put numbers on each sample for identification and take care to match each sample with the corresponding mass. Open the sachets, remove the content using a small brush, etc., and weigh each empty sachet accurately. Subtract the mass of each empty sachet from the mass of the matching sample to obtain the mass of the content individually. When the average mass of the content is calculated by measuring the mass of the content from 20 samples, if the number of samples in which mass deviates > 10% from the average mass is ≤ 2 and the number of samples in which mass deviates > 25% from the average mass is zero, such preparation is considered to meet the requirements.

B) Tablets

When the average mass is calculated by accurately weighing 20 samples, if the number of samples of which mass deviates from the average mass by the value listed below, if any, is ≤ 2 and the number of samples of which mass deviates from the average mass by double the value listed below is zero, such preparation is considered to meet the requirements.

Average mass (g)	Variation (%)
< 0.12	10
≥ 0.12 and < 0.3	7.5
≥ 0.3	5

3) Suppositories

When the average mass is calculated by accurately weighing 20 samples, if the variation between the average value and the mass of each sample is ≤ 5% and the number of samples in which mass deviates from the average mass by > 5% and ≤ 7.5% is ≤ 2, such preparation is considered to meet the requirements.

4) Injections (to be dissolved or suspended before use)

Take 10 samples, remove the labels, wash the exterior, air-dry, dry them completely in a desiccator to get a constant mass, and weigh their mass accurately. Open the containers carefully to remove the content, wash all parts of each container with water and ethanol, air-dry, then dry them completely in a desiccator to get a constant mass, and weigh them accurately. The difference between the mass before and after is taken as the mass of the content. Calculate the average mass of the contents of 10 injections and the variation between the mean value and each mass. To meet the requirements, the number of outliers by the following values is NMT 1 and variation of more than 2 times the following values is not observed.

Average mass (g)	Variation (%)
< 0.015	15
≥ 0.015 and < 0.12	10
≥ 0.12 and < 0.3	7.5
≥ 0.3	7

5) Capsules

A) Hard Capsules

When the average mass is calculated by accurately weighing 20 samples, if the variation between the average value and the mass of each sample is ≤ 10%, the preparation is considered to meet the requirements. If the difference exceeds 10% in any of the samples, take another 20 samples and weigh the mass of each capsule accurately. At this time, put numbers on each capsule for identification and take care to match each capsule with the corresponding mass. Open the capsules, remove the content using a small brush, etc., and weigh each empty capsule shell accurately. Subtract the mass of each empty shell from the mass of the matching capsule to obtain the mass of the content individually. When the average mass of the content is calculated by measuring the mass of the content from 20 capsules, if the number of samples in which mass deviates > 10% from the average mass is ≤ 2 and the number of samples in which mass deviates > 25% from the average mass is zero, such preparation is considered to meet the requirements.

B) Soft Capsules

Take 20 capsules and weigh the mass of each capsule accurately. At this time, put numbers on each capsule for identification and take care to match each capsule with the corresponding mass. Open the capsules, remove the content by rinsing them with a volatile solvent, such as ether, remove the remaining solvent from the empty capsule shell by lightly dabbing with a filter paper, etc., and leave the shells at ordinary temperature to further remove the remaining solvent. At this time, it is necessary to prevent the capsule shell from absorbing moisture or drying out. Weigh the mass of each empty shell accurately, and subtract the mass of each empty shell from the mass of the matching capsule to obtain the mass of the content individually. When the average mass of the content is calculated from 20 capsules, if the number of samples in which mass deviates > 10% from the average mass is ≤ 2 and the number of samples in which mass deviates > 25% from the average mass is zero, such preparation is considered to meet the requirements. When the number of capsules in which mass variation is > 10% and ≤ 25% is 3 to 6, repeat the test with additional 40 capsules in the same manner. When the average mass is calculated from 60 capsules in total, if the number of samples in which mass deviates > 10% from the average mass is ≤ 6 and the number of samples in which mass deviates > 25% from the average mass is zero, such preparation is considered to meet the requirements.

6) Troches

When the average mass is calculated by precisely weighing 20 samples, if the number of samples in which mass deviates > 10% from the average mass, if any, is ≤ 2 and the number of samples in which mass deviates > 20% from the average mass is zero, such preparation is considered to meet the requirements.

Table 2. Definition of Terms, etc.

Variable	Definition	Conditions	Value
$\overline{X}$	Mean content of individual samples expressed as a% for the labeled amount (x_1, x_2, …, x_n)		
$x_1, x_2, \cdots, x_n$	Content of the drug substance contained in each sample tested (expressed as a% for the labeled amount)		
n	Sample size (number of dosage units in a sample)		
k	Acceptability constant	If the number of samples, n =10, then	2.4
		If the number of samples, n =30, then	2.0
s	Simple standard deviation		$\sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{X})^2}{n-1}}$
RSD	Relative standard deviation (standard deviation expressed as% for the mean value)		$\frac{100s}{\overline{X}}$
M To be applied when $T \leq 101.5$	Reference value	$98.5\% \leq \overline{X} \leq 101.5\%$	$M = \overline{X}$ $(AV = ks)$
		$\overline{X} < 98.5\%$,	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		$\overline{X} > 101.5\%$,	$M = 101.5\%$ $(AV = \overline{X} - 101.5 + ks)$
M To be applied when $T > 101.5$	Reference value	$98.5 \leq \overline{X} \leq T$	$M = \overline{X}$ $(AV = ks)$
		$\overline{X} < 98.5\%$	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		$\overline{X} > T$	$M = T\%$ $(AV = \overline{X} - T + ks)$
Acceptance Value (AV)			General formula: $\lvert M - \overline{X} \rvert + ks$ [Calculations are specified above for the different cases.]
$L1$	Upper limit of the acceptance value		$L1$= 15.0 Unless otherwise specified.
$L2$	Upper limit of the acceptance deviation from M for the content in each dose unit	The lower and upper limits of the content in each dose unit are 0.75 M and 1.25 M, respectively ($L2$=25.0)	$L2$ = 25.0 Unless otherwise specified.
T	The target content, T is 100.0%, unless otherwise specified in the monograph.		

Viscosity
점도측정법

The Viscosity is a method used to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a certain direction and there is a difference in velocity in the direction perpendicular to the flow, an internal frictional force is generated on both sides of a plane parallel to the flow. This property of liquid is called viscosity. The internal frictional force per unit area on the plane parallel to the flow is called the shear stress, and the velocity slope in the direction perpendicular to the flow is called the shear rate. A liquid in which the shear stress is proportional to its shear rate is called a Newtonian liquid. The proportionality constant, η, which is an intrinsic constant of a liquid at a certain temperature, is called viscosity. The unit of viscosity is Pascal second(Pa·s), but milli-Pascal second (mPa·s) is generally used.

A liquid in which shear stress is not proportional to its shear rate is called a non-Newtonian liquid, and the viscosity of such liquid is called apparent viscosity in that it changes in various ways depending on the shear rate. In this case, the value obtained by dividing the shear stress by the corresponding shear rate is the apparent viscosity, and if the relationship between the shear rate and the apparent viscosity is obtained, the flow characteristics of a given non-Newtonian liquid can be found.

The quotient obtained by dividing the viscosity, η, by the density of the liquid at the same temperature is called the kinematic viscosity, ν, the unit of which is square meters per second (m^2/s), but square millimeters per second (mm^2/s) is more commonly used. The viscosity of a liquid is determined using one of the following methods.

Method I. Viscosity measurement by capillary tube viscometer

This method is used to measure the viscosity of Newtonian liquids, in which the time, t (s), required for a certain volume of liquid to flow down through the capillary tube is measured, and then the kinematic viscosity, ν, is calculated using the following equation.

$$\nu = K \cdot t$$

To determine Viscosity, η, the density of the liquid sample,

ρ (g/mL), is measured again at the same temperature and the following equation is used for the calculation.

$$\eta = \nu \cdot \rho = K \cdot t \cdot \rho$$

K (mm^2/s^2) represents the constant of the viscometer, which is predetermined using the standard solutions for calibrating viscometers. For a viscometer to be used for measuring samples having a viscosity similar to water, water is usually used as the standard solution. The kinematic viscosity of water is 1.0038 mm^2/s at 20 °C. For a viscometer to be used for measuring samples having a relatively higher viscosity, the standard solutions for calibrating viscometers are used.

The intrinsic viscosity, $[\eta]$ (dL/g), of a polymer solution can be determined by extrapolating the concentration of the straight line obtained by measuring the concentration dependence of the viscosity of a liquid that contains the polymeric material to zero. The intrinsic viscosity indicates the degree of diffusion of a polymer in a liquid (test solution) and also serves as a reference standard for molecular weight. The intrinsic viscosity is calculated using the following equation by measuring the flow time, t, of the test solution with the concentration c (g/dL) and the flow time, t_0, of the solvent.

$$[\eta] = \lim_{c \to 0} \frac{ln\frac{t}{t_0}}{c} \text{ or } [\eta] = \lim_{c \to 0} \frac{(\frac{t}{t_0})-1}{c}$$

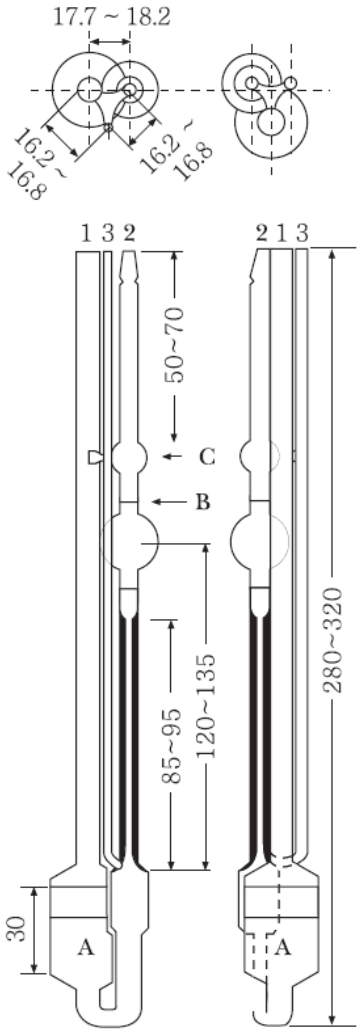
However, when the concentration dependency of $\{(t/t_0)-1\}/c$ is not that large, the value of $\{(t/t_0)-1\}/c$ obtained from the test solution specified in the monograph can be used as the intrinsic viscosity for a given substance.

The time to flow down is measured using the following apparatus and procedure.

Apparatus To measure the kinematic viscosity of a liquid in the range of 1 to 100000 mm^2/s, use an Ubbelohde-type viscometer, as shown in Figure 1. The table below shows the general relationship between the internal diameter of the capillary tube and the range of kinematic viscosity suitable for measurement. Viscometers other than those listed in this table can be used, but in such case, select a viscometer based on the internal diameter of the capillary tube to ensure that the time to flow down is 200 to 1000 seconds.

Procedure Put in the liquid sample gently from tube 1, and then carefully place the viscometer vertically so that the meniscus of the liquid sample comes between the two gauge lines of bulb A. Put the viscometer in a constant-temperature bath at the temperature (± 0.1 °C)specified in the monograph so that bulb C is completely submerged in water, hold it vertically, and leave it for about 20 minutes until the sample reaches the specified temperature. Cover tube 3 with a finger and gently apply suction from the upper end of tube 2, while preventing air bubbles from entering tube 2, to raise the liquid level to the center of bulb C. Then, stop the suction, open the inlet of tube 3, and immediately cover the inlet of tube 2. After confirming that the liquid column at the bottom of the capillary tube is broken, open the inlet of tube 2 and measure the time, t (sec), for the liquid level to flow down from the upper to the lower gauge line of bulb B.

Determine the viscometer constant, K, in advance, using the standard solutions for calibrating viscometers in the same way. However, at this time, follow the temperature condition specified in the monograph.



* The figures are in mm.

Figure 1. **Diagram of a capillary tube viscometer**

Table. Specifications of Ubbelohde-type viscometers

Approximate viscometer constant (K) (mm^2/s^2)	Internal diameter of capillary tube (mm) [Permissible tolerance : ± 10%]	Volume of bulb B (mL) [Permissible tolerance : ± 10%]	Measuring range of kinetic viscosity (mm^2/s)
0.005	0.46	3.0	1 - 5
0.01	0.58	4.0	2 - 10
0.03	0.73	4.0	6 - 30
0.05	0.88	4.0	10 - 50
0.1	1.03	4.0	20 - 100
0.3	1.36	4.0	60 - 300
0.5	1.55	4.0	100 - 500
1.0	1.83	4.0	200 - 1000
3.0	2.43	4.0	600 - 3000
5.0	2.75	4.0	1000 - 5000
10.0	3.27	4.0	2000 - 10000
30.0	4.32	4.0	6000 - 30000
50.0	5.20	5.0	10000 - 50000
100	6.25	5.0	20000 - 100000

Method II. Viscosity measurement by rotational viscometer

As a method used for measuring Newtonian liquids or non-

Newtonian liquids, this measurement method utilizes the principle of detecting the force (torque) acting on the rotor rotating at a constant angular velocity in an ordinary liquid through the extent of torsion of a spring, and converting it into viscosity.

The viscosity of a liquid sample is measured using the following apparatuses and procedures.

Apparatus To measure viscosity, use any one of the following viscometers.

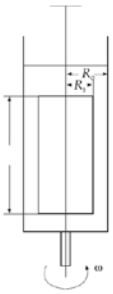


Figure 2a
Coaxial double cylinder type rotational viscometer

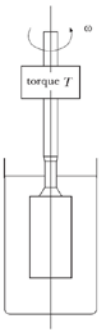


Figure 2b
Single cylinder type rotational viscometer

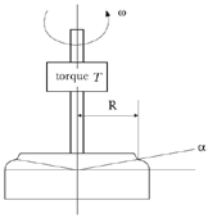


Fig 2c
Cone-and-plate type rotational viscometer

A) Coaxial double cylinder type rotational viscometer

The coaxial double cylinder rotational viscometer measures the torque or angular velocity transmitted between cylinders by a liquid when the outer or inner cylinder sharing the same central axis is rotated after the gap between the outer and inner cylinders is filled with the liquid.

As shown in Fig. 2a, the inner cylinder is hung by a wire for which the twist constant is designated as *k*. The radii of the inner and outer cylinders are designated as R_i and R_o, respectively, and the length of the inner cylinder immersed in a liquid is designated as l. When a liquid is introduced into the outer cylinder and rotated at a constant angular velocity, ω, the inner cylinder is also forced to rotate due to the viscosity of the liquid, but since a torque, *T*, is generated in the wire, the inner cylinder rotates by θ to balance. At this time, $T = k \cdot \theta$, and by measuring the relationship between ω and θ, the viscosity of the liquid, η, can be calculated using the following equation. In the same way, the same equation holds even when the inner cylinder is rotated.

$$\eta = \frac{100T}{4\pi\, l\, \omega}\left[\frac{1}{{R_i}^2} - \frac{1}{{R_o}^2}\right]$$

η: Viscosity of the sample (mPa·s)
π: Circumference/diameter ratio
l: Length of the inner cylinder (cm)
ω: Angular velocity (rad/s)
T: Torque acting on the cylinder surface (10^{-7} N·m)
R_i: 1/2 of outer diameter of the inner cylinder (cm)
R_o: 1/2 of internal diameter of the outer cylinder (cm)

B) Single cylinder type rotational viscometer

The single cylinder rotational viscometer is a viscometer that measures the torque generated when a cylinder in a liquid is rotated at a constant angular velocity. The apparatus shown in Fig. 2b is used. The apparatus constant, K_B, is predetermined experimentally in advance using the standard solutions for calibrating viscometers to calculate the viscosity of the liquid, η, according to the following equation.

$$\eta = K_B \cdot \frac{T}{\omega}$$

η: Viscosity of the liquid sample (mPa·s)
K_B: Apparatus constant of the viscometer (rad/cm^3)
ω: Angular velocity (rad/s)
T: Torque acting on the cylinder surface (10^{-7} N·m)

C) Cone-and-plate type rotational viscometer

The cone-and-plate type rotational viscometer is used to measure the torque and angular velocity received by the other side while one side is rotated after the gap between a flat disc and a cone with a large vertical angle that share the same rotation axis is filled with a liquid. The apparatus shown in Fig. 2c is used.

When a liquid is introduced into the gap between a cone and a flat disc at the angle α and the cone or the flat disc is rotated at a constant angular velocity, the torque received by the flat disc or the cone and the corresponding angular velocity at the time when a steady state is reached is measured to calculate the viscosity of the liquid, η, according to the following equation.

$$\eta = 100 \times \frac{3\alpha}{2\pi R^3} \times \frac{T}{\omega}$$

η: Viscosity of the sample (mPa·s)
π: Circumference/diameter ratio
R: Radius of the cone (cm)
α: Angle between the flat disc and the cone (rad)
ω: Angular velocity (rad/s)
T: Torque acting on the cylinder surface (10^{-7} N·m)

Procedure Install the viscometer in such a way that its rotating axis is perpendicular to the horizontal plane. After filling the viscometer with a sufficient amount of the test solution for measurement, leave it until it reaches the temperature specified in the monograph. If it is desired to measure the viscosity of the sample with a precision of less than 1%, it is necessary to keep the temperature control of the measuring system within ± 0.1 ℃. After confirming that the temperature of the test solution has reached the designated value, start the viscometer. Once the rotation of the viscometer reaches a steady state and the indicated value on the viscometer corresponding to the number of rotations or the torque becomes stabilized, read the indicated value and calculate the viscosity, η, with the equation appropriate for each viscometer used. Also, predetermine and check the apparatus constant of the viscometer in advance using the standard solutions for calibrating viscometers to validate the operating procedure.

For non-Newtonian liquids, repeat the procedure for measuring the apparent viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another by applying a constant rotational speed or a constant torque, and from the series of these measurements, determine the relationship (flow curve) between the sheer rate and the sheer stress of the sample.

To calibrate viscometers, use water and the standard solutions for calibrating viscometers. These are used to determine or verify the apparatus constant of the rotational viscometer. In addition, it is recommended to recalibrate the viscometer regularly to ensure that the specified precision of measurement is guaranteed.

Vitamin A assay
비타민 A 정량법

The Vitamin A Assay is a method of quantifying vitamin A in the retinol acetate, retinol palmitate, vitamin A oil, cod liver oil and other preparations using the absorbance determination method.

Generally, it is necessary to perform an appropriate pretreatment depending on the type of preparation to be analyzed or the substance that hinders the quantification.

One vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.3 μg of vitamin A (alcohol form).

Reagents

In this method, 2-propanol and ether described below are used.

When tested according to the Ultraviolet-visible Spectroscopy in comparison with water, 2-propanol has absorbance of NMT 0.05 at the wavelength of 300 nm, and has absorbance of NMT 0.01 at the wavelength of 320 to 350 nm. If necessary, it should be purified by distillation.

When using ether, distill and discard it around 10% of the first and last portions.

Procedure

Perform the procedure as quickly as possible. Avoid contact with the air or other oxidants and use a light resistant container.

Method 1 is used unless otherwise specified in the monograph, but Method 2 is used for cases that are not suitable for the conditions of Method 1.

Method 1 Precisely weigh about 0.5 g of the sample and dissolve it in 2-propanol to make the final volume exactly 250 mL. Test this solution using the Ultraviolet-visible Spectroscopy. Accurately dilute the solution with 2-propanol to make the absorbance about 0.5 at the wavelength 326 nm, and use the resulting solution as the test and standard solution. Then, measure the maximum absorption wavelength. Also, measure the absorbance at the wavelengths of 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm, and obtain the ratio of absorbance at each wavelength when the absorbance at the wavelength 326 nm is 1.000. If the absorption maximum appears at wavelengths 325 to 328 nm and the ratio of absorbance at each wavelength is within ± 0.030 specified in the table, calculate the vitamin A unit in 1 g of the sample using the absorbance A at the wavelength of 326 nm.

$$\text{Units of vitamin A in 1 g} = E_{1\text{cm}}^{1\%}\ (326\text{ nm}) \times 1900$$

$$E_{1\text{cm}}^{1\%}\ (326\text{ nm}) = \frac{A}{M} \times \frac{V}{100}$$

V: Total volume (mL) of the test solution

M: Amount (g) of sample in *V* mL of the test solution

λ (nm)	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
326	1.000	1.000
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

For identification of retinol acetate and retinol palmitate, perform the following the Identification.

Identification Prepare the test and standard solution, retinol acetate standard solution and retinol palmitate standard solution by weighing the sample, retinol acetate standard solution, and retinol palmitate standard solution at the amount that corresponds to 15,000 vitamin A units each and dissolving in 5 mL of petroleum ether, respectively. Test these solutions using thin-layer chromatography. Spot 5 μL each of the test and standard solution, retinol acetate standard solution and retinol palmitate standard solution onto a thin layer plate made with silica gel for thin-layer chromatography. Then, develop by around 10 cm using the mixture of cyclohexane and ether (12:1) as a developing solvent and dry the thin layer plate in the air. On the dried plate, evenly spray the antimony trichloride (III) reagent. Then, compare the locations of the blue main spots obtained from the test and standard solution, retinol acetate standard solution and retinol palmitate standard solution.

Perform the procedure according to Method 1, and if there is no absorption maximum at the wavelength of 325 to 328 nm or the ratio of absorbance is outside of the ± 0.030 specified in the table, follow Method 2.

Method 2 Unless otherwise specified, precisely weigh the sample in the amount that corresponds to at least 500 vitamin A units and contains NMT 1 g of oil, and place it into a flask. Add 30 mL of aldehyde-free ethanol and 1 mL of pyrogallol ethanol solution (1 in 10) into the flask. Then, add 3 mL of potassium hydroxide solution (9 in 10) and attach a reflux condenser to the flask. Heat it for 30 minutes on a water bath to saponify the content. Quickly cool the content to ordinary temperature, add 30 mL of water, and transfer it to the separatory funnel A. Wash the flask sequentially with 10 mL of water and then 40 mL of ether. Transfer the solution used for washing to separatory funnel A and shake well, and allow it to stand. Take the water layer and transfer it to separatory funnel B. Wash the flask with 30 mL of ether and transfer the solution used for washing to separatory funnel B, then shake it and extract it. Take the water layer separately to put it into the flask and combine the ether layer into separatory funnel A. Add the water layer into separatory funnel B and also add 30 mL of ether, then shake it and extract it. Combine the ether layer into separatory funnel A. To this, add 10 mL of water and gently invert the funnel 2 to 3 times. Then, allow to stand, and discard the separated water layer. Again, perform washing with 50 mL of water 3 times. At each time of washing, shake it more strongly than the previous time of washing. Perform washing with 50 mL of water several times until the solution used for washing does not show a color reaction with the phenolphthalein reagent. Once the washing is complete, allow it stand for 10 minutes. Eliminate the water as best as possible and transfer the ether extract solution to an Erlenmeyer flask. Wash it twice with 10 mL of ether and then add 5 g of sodium sulfate anhydrous and shake to mix. Incline the Erlenmeyer flask to transfer the mixed ether extract to an eggplant flask. Wash the remaining sodium sulfate with 10 mL of ether no less than twice and combine the solution used for washing to the flask. Shake the ether extract on a water bath at 45°C, concentrate it with an aspirator to about 1 mL, promptly add 2-propanol for vitamin A quantification, and dilute so that the concentrate contains exactly 6 to 10 vitamin A units per 1 mL. Use the resulting solution as the test and standard solution. Test this solution according to the Ultraviolet-visible Spectroscopy. Measure the absorbance A_1, A_2 and A_3 at the wavelengths 310 nm, 325 nm and 334 nm.

Units of vitamin A in 1 g of the sample

$$= E_{1cm}^{1\%} \ (325\ nm) \times 1830$$

$$E_{1cm}^{1\%} \ (325\ nm) = \frac{A_2}{M} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f: Correction factor
V: Total volume (mL) of the test solution
M: Amount (g) of sample in *V* mL of the test solution

Water
수분측정법 (칼피셔법)

The Water is a method used to quantify the water content in sample materials. This is achieved through a quantitative reaction between water, iodine and sulfur dioxide in the presence of lower alcohol such as methanol and organic base like pyridine as shown in the following reaction equation.

$$I_2 + SO_2 + 3C_5H_5N + CH_3OH + H_2O \rightarrow$$
$$2(C_5H_5N{+}H)I^- + (C_5H_5N{+}H)^-OSO_2OCH_3$$

There are two measurement methods: volumetric titration and coulometric titration. Volumetric titration involves dissolving the iodine required for the reaction in a test solution for water determination (from this point forward, referred to as "Karl Fischer TS") and measuring the water content based on the amount of iodine consumed during reaction with water in the sample. Coulometric titration involves generating the iodine by electrolyzing Karl Fischer TS containing iodide ions and measuring the water content based on the quantity of electricity consumed in electrolysis using the quantitative reaction of iodine with water.

$$2\ I^- \rightarrow I_2 + 2\ e^-$$

Volumetric titration

Apparatus

The apparatus generally consists of automatic burette, titration flask, stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current. Since the Karl Fischer TS is extremely hygroscopic, the apparatus should be protected from absorbing moisture from outside. Desiccants such as silica gel or calcium chloride for water determination are used to prevent moisture.

Reagents

A) Chloroform for water determination

Add 30 g of synthetic zeolite for moisture absorption to 1000 mL of chloroform, seal tightly, and allow to stand for about 8 hours with occasional shaking. After leaving it for about 16 hours, collect the clear chloroform layer. Store away from moisture. The water content in this solution should not be more than 0.1 mg per mL.

B) Methanol for water determination

Add 30 g of synthetic zeolite for moisture absorption (or moisture elimination) to 1000 mL of methanol, seal tightly, and allow to stand for about 8 hours, shaking occasionally. After leaving it for about 16 hours, collect the clear methanol layer. Store away from moisture. The water content in this solution should not be more than 0.1 mg per mL.

C) Propylene carbonate for water determination

Add 30 g of synthetic zeolite for moisture absorption to 1000 mL of propylene carbonate, seal tightly, and allow to stand for about 8 hours with occasional shaking. After leaving it for about 16 hours, collect the clear propylene carbonate layer. Store away from moisture. The water content in this solution should not be more than 0.3 mg per mL.

D) Diethylene glycol monoethyl ether for water determination

Add 30 g of synthetic zeolite for moisture absorption to 1000 mL of diethylene glycol monoethyl ether, seal tightly, and allow to stand for about 8 hours with occasional shaking. After leaving it for about 16 hours, collect the clear layer of diethylene glycol monoethyl ether. Store away from moisture. The water content of this solution should not be more than 0.3 mg per mL

E) Pyridine for water determination

Add potassium hydroxide or barium oxide to pyridine, seal tightly, and allow to stand for several days with occasional shaking. Distill the pyridine while obstructing moisture and store the distillate away from moisture. The water content in this solution should not be more than1 mg per mL.

F) Imidazole for water determination

Store imidazole away from moisture. The water content in the imidazole should not be more than 1 mg per 1 g.

G) 2-Methylaminopyridine for water determination

Distill 2-methylaminopyridine as it is while obstructing moisture and store the distillate away from moisture. The water content in this solution should not be more than1 mg per mL.

Preparation method of test solution and standard solutions

A) Karl Fischer TS

Karl Fischer TS is preserved in a cold place while protecting it from light and moisture.

① Preparation Prepare the Karl Fischer TS according to one of the following methods. Additives may be added to improve the performance, including for stability, after confirming that equivalent results to those obtained from the specified method can be achieved.

Preparation method 1 Dissolve 63 g of iodine in 100 mL of pyridine for water determination, cool the solution with ice and pass dry sulfur dioxide through. When the increased amount reaches 32 g, add chloroform for water determination or methanol for water determination to make 500 mL, and allow to stand for more than 24 hours before use.

Preparation method 2 Dissolve 102 g of imidazole for water determination in 350 mL of diethylene glycol monoethyl ether for water determination and cool the solution with ice. While maintaining the temperature of the solution at 25 to 30 ℃, pass dry sulfur dioxide through. When the increased amount reaches 64 g, add 50 g of iodine to dissolve it, and allow to stand for more than 24 hours before use.

Preparation method 3 Dissolve 81 g of 2-methylaminopyridine for water determination in 180 mL of propylene carbonate for water determination or diethylene glycol monoethyl ether for water determination and cool the solution with ice. Pass dry sulfur dioxide through 220 mL of propylene carbonate for water determination. When the amount of the increase reaches 32 g, add it to the cooled solution, add 36 g of iodine, dissolve it, and allow to stand for more than 24 hours before use.

② Standardization Perform standardization of Karl Fischer TS before use as it changes over time. According to the procedure, put an appropriate amount of methanol for water determination into a dry titration flask and add the Karl Fischer TS dropwise to the endpoint in advance to make the inside of the flask anhydrous. Then, accurately weigh 30 mg of water, add it immediately to the titration flask, and titrate to the endpoint with

Karl Fischer TS while stirring vigorously. Calculate f (mg/mL), the milligrams of water (H_2O) equivalent to 1 mL of Karl Fischer TS, using the following equation:

$$f\,(\mathrm{mg/mL}) = \frac{\text{Amount of water (H 2O) taken (mg)}}{\text{Volume of Karl Fischer TS consumed for titrat ion of water } (H_2O)\ (\mathrm{mL})}$$

B) Water-methanol standard solution

Store this standard solution in a cold place away from light and moisture.

① **Preparation** Put 500 mL of methanol for water determination into a dry 1000 mL flask, add 2.0 mL of water, and add methanol for water determination to make it up to 1000 mL.

② **Standardization** Perform standardization of this solution immediately after the standardization of Karl Fischer TS. According to the procedure, put an appropriate amount of methanol for water determination into a dry titration flask and add the Karl Fischer TS dropwise to the endpoint in advance to make the inside of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS to this solution and titrate it with the prepared standard water/methanol solution to the endpoint. Calculate f' (mg/mL), which is the mg of water (H_2O) in 1 mL of the water-methanol standard solution, using the following equation.

$$f'\ (\mathrm{mg/mL}) = \frac{f\ (\mathrm{mg/mL}) \times 10\ (\mathrm{mL})}{\text{Volume of the standard water} - \text{methanolsolution consumed for titration (mL)}}$$

Procedure

In principle, titration should be performed at the same temperature as the standardization of Karl Fischer TS while obstructing moisture. Immerse a pair of platinum or double platinum electrodes in the titrant, apply a minute voltage between the electrodes by adjusting the variable resistance, and measure the change in current (μA) when dropping the Karl Fischer TS. As the titration progresses, the current in the circuit changes significantly, but returns to its initial state within a few seconds. When the endpoint of titration is reached, this current change continues for a period of time (usually 30 seconds or more). This state is considered as the endpoint of the titration (amperometric titration with constant polarization voltage). Alternatively, measure the potential difference (mV) that changes when dropping the Karl Fischer TS while allowing a minute current to flow between the electrodes. As the titration progresses, the value of the voltmeter in the circuit drops rapidly from a polarization state of several hundred mV to a non-polarization state, but returns to its initial state within a few seconds. When the endpoint of titration is reached, the non-polarization state continues for a certain period of time (usually 30 seconds or more). This state is considered the endpoint of the titration (potentiometric titration with constant polarization current). However, when amperometric titration with constant polarization voltage is used in a back titration, the needle of the microammeter goes off-scale in the presence of excessive Karl Fischer TS, but quickly returns to the initial position when the titration system reaches the endpoint. In potentiometric titration with constant polarization current, the needle of the millivoltmeter stays in the initial position in the presence of excessive Karl Fischer TS. Finally, a constant voltage is displayed when the titration system reaches the endpoint.

Unless otherwise specified, titration using the Karl Fischer TS is performed by direct titration or back titration. The endpoint of the titration is usually easier to determine when back titration is used.

A) Direct titration

Unless otherwise specified, proceed as follows. Put an appropriate amount of methanol for water determination into a dry titration flask and drop the Karl Fischer TS until it reaches the endpoint in advance to make the inside of the flask anhydrous. Then, accurately weigh a sample containing 5 to 30 mg of water, quickly add it to the titration flask, stir it to dissolve, and titrate with the Karl Fischer TS while stirring vigorously until it reaches the endpoint. If the sample does not dissolve in the solvent, powder it quickly, accurately weigh the sample containing 5 to 30 mg of water, add it immediately to the titration flask, stir for 5 to 30 minutes while avoiding moisture, and then titrate with vigorous stirring. In particular, if the sample does not dissolve in the solvent or interferes with the Karl Fischer reaction, the sample can be heated with a separate water evaporator. Then, water in the sample can be introduced into the titration flask, with nitrogen as the carrier gas. Titration must be performed under low humidity. However, if the effect of humidity during the test cannot be avoided, e.g., due to an extended period of titration, perform correction through a blank test using the same procedure as for measuring the sample.

$$\text{Amount of water } (H_2O)\ (\%) = \frac{V\ (\mathrm{mL}) \times f\ (\mathrm{mg/mL})}{\text{Sample weight (mg)}} \times 100$$

V: Volume of Karl Fischer TS consumed in the titration

B) Back titration

Unless otherwise specified, proceed as follows. Put an appropriate amount of methanol for water determination into a dry titration flask, and drop the Karl Fischer TS until it reaches the endpoint in advance to make the inside of the flask anhydrous. Then, accurately weigh a sample containing 5 to 30 mg of water, quickly transfer it to a titration flask, add some of the excess Karl Fischer TS, stir to dissolve, and titrate with water/methanol standard solution until it reaches the endpoint while stirring vigorously. If the sample is insoluble in the solvent, powder it quickly, accurately weigh the mass, immediately transfer it to a titration flask, add a certain amount of the excess Karl Fischer TS, stir for 5 to 30 minutes while avoiding moisture, and then titrate while stirring vigorously.

$$\text{Amount of water } (H_2O)\ (\%) = \frac{V_0\ (\mathrm{mL}) \times f\ (\mathrm{mg/mL}) - V\ (\mathrm{mL}) \times f'\ (\mathrm{mg/mL})}{\text{Sample weight (mg)}} \times 100$$

V_0: Amount of Karl Fischer TS

V: Volume of the standard water/methanol solution consumed in the titration

Coulometric titration

Apparatus The apparatus usually consists of a titration flask with an electrolytic cell for iodine generation, a stirrer, and a potentiometric titration system with constant current. The iodine generator consists of an anode and a cathode separated by a diaphragm. The anode is immersed in an anolyte for water determination, while the cathode is in a catholyte for water determination. Generally, both electrodes use a platinum mesh. Since the anolyte and catholyte for water determination are highly hygroscopic, the apparatus should prevent moisture absorption from the outside. Desiccants such as silica gel or calcium chloride for water determination are used to prevent moisture.

Preparation method of anolyte and catholyte solutions for water determination Anolyte and catholyte for water determination are a set of reagents, and are prepared using one of the following methods:

Preparation method 1

① Anolyte for water determination

Dissolve 40 g of 1,3-di (4-pyridyl) propane and 30 g of diethanolamine in approximately 200 mL of methanol for water determination, and pass through dry sulfur dioxide. Then, when the increased amount reaches 25 g, add 50 mL of propylene carbonate to dissolve 6 g of iodine, and add methanol for water determination to make it up to 500 mL. Spot water until the color of the solution changes from brown to yellow.

② Catholyte for water determination

Dissolve 24 g of diethanolamine hydrochloride in 100 mL of methanol for water determination.

Preparation method 2

① Anolyte for water determination

Dissolve 40 g of 1,3-di (4-pyridyl) propane and 30 g of diethanolamine in about 200 mL of methanol for water determination, and pass through dry sulfur dioxide. Then, when the increased amount reaches 25 g, add 50 mL of propylene carbonate to dissolve 6 g of iodine, and add methanol for water determination to make 500 mL. Spot water until the color of the solution changes from brown to yellow.

② Catholyte for water determination

Dissolve 30 g of choline chloride in methanol for water determination to make 100 mL.

Preparation method 3

① Anolyte for water determination

Dissolve 100 g of diethanolamine in 900 mL of methanol for water determination or a mixture of methanol for water determination and chloroform for water determination (3 : 1) and cool it down. Then, pass through dry sulfur dioxide. When the increased amount reaches 64 g, add 20 g of iodine to dissolve it, and drop water until the color of the solution changes from brown to yellow.

② Catholyte for water determination

Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitroethane (4:1).

Procedure After adding the anolyte for water determination to the titration flask, immerse a pair of platinum electrodes or double platinum electrodes of the potentiometric titration system with constant current in that solution. Separately, immerse the iodine generator filled with the anolyte for water determination into the anolyte for water determination. Apply an electrolytic current in advance to make the inside of the titration flask anhydrous. Next, accurately weigh a sample containing 0.2 to 5 mg of water, quickly add it to the titration flask, stir it to dissolve, and titrate it to the endpoint with vigorous stirring. If the sample is insoluble in anolyte, powder it quickly. Then, accurately weigh the sample containing 0.2 to 5 mg of water, add it immediately to the titration flask, stir for 5 to 30 minutes while avoiding moisture, and then titrate with vigorous stirring. If the sample does not dissolve in the solvent or interferes with the Karl Fischer reaction, the sample can be heated with a separate water evaporator. Then, the water in the sample can be introduced into the titration flask with nitrogen as the carrier gas. Measure the quantity of electricity (C) [current (A) × time (s)] consumed for iodine generation from the start of the titration to the endpoint, and determine the water content (%) in the sample by using the following equation. The Titration must be performed under low humidity. However, if the effect of humidity during the test cannot be avoided, e.g., due to an extended period longtime of titration, perform correction through a blank test using the same procedure as for measuring the sample.

$$\text{Amount of water } (H_2O)\ (\%) = \frac{\text{Quantity of electricity consumed for generating iodine (C)}}{10.72 \times \text{mass of sample (mg)}} \times 100$$

10.72: Quantity of electricity corresponding to 1 mg of water (H_2O) (C/mg)

Reference Standards, Reagents and Test Solutions, Standard Solutions for Volumetric Analysis, Standard Solutions, Matching Fluids for Color, Optical Filters for Wavelength and Transmission Rate Calibration, Measuring Instruments and Appliances, Sterilization and Aseptic Processing

표준품, 시약·시액, 용량분석용표준액, 표준액, 색의 비교액, 파장 및 투과율 보정용 광학필터, 계량기·용기, 멸균법 및 무균조작법

1) Reference Standards
2) Reagents and Test Solutions
3) Standard Solutions for Volumetric Analysis
4) Standard Solutions
5) Matching Fluids for Color
6) Optical Filters for Wavelength and Transmission Rate Calibration
7) Measuring Instruments and Appliances
8) Sterilization and Aseptic Processing

Reference standards are a highly purified and well-characterized material suitable to test the identification, quality, and purity of substances for Chemical, pharmaceutical, and medicinal products.

Reagents are substances or compounds, used in the tests defined in the Korean Pharmacopoeia. The phrase "as specified in the monograph" means that the reagent confirms to the requirements for the corresponding monograph.

Test solutions are solutions prepared for the tests defined in the Korean Pharmacopoeia.

Standard solutions for volumetric analysis are solutions having known concentrations and intended primarily for use in quantitative analysis.

Standard solutions are solutions used as a basis for comparison in the tests defined in the Korean Pharmacopoeia.

Matching fluids for color are used as a reference for comparing colors in the tests defined in the Korean Pharmacopoeia.

Measuring instruments are instruments or Appliances used for measurements in the tests defined in the Korean Pharmacopoeia.

Appliances are a device designed to make the conditions as constant as possible in the test defined in the Korean Pharmacopoeia.

1) Reference Standards

Acanthoside D, Acarbose, Acebutolol Hydrochloride, Aceclofenac, Acetaldehyde, Acetaminophen, Acetamiprid, Acetylcholine Chloride, Acetylcysteine, Acetylspiramycin II, Acetylspiramycin, Aconitine, Acrinol Hydrate, Acrinol, Acyclovir, Adenosine, Albendazole, Albiflorin, Aldioxa, Aldrin, Alendronate Sodium Trihydrate, Alfacalcidol, Alfuzosin Hydrochloride, Alimemazine Tartrate, Allantoin, Allopurinol, Almagate, Aloeemodin, Alprostadil, Amantadine Hydrochloride, Amidotrizoic Acid, Amikacin Sulfate, Amikacin, p-Aminobenzoyl glutamic acid, Aminobutanol, Aminocaproic Acid, 3-Aminopent-4-ene-11-Dicarboxylic Acid, Amitriptyline Hydrochloride, Amlodipine Besylate, Amoxicillin, Amphotericin B, Ampicillin Sodium, Ampicillin, Amygdalin, Anesaldehyde, Anetole, Anhydrous Caffeine, Anhydrous Lactose, Anthralin, Arbekacin Sulfate, Arctigenin, Arecoline Hydrobromide, Arginine Hydrochloride, Arginine, Aristolokinic Acid, Arotinolol Hydrochloride, Ascorbic Acid, Aspirin, Aspoxicillin, Astromicin Sulfate, Atenolol, Atorvastatin Calcium, Atracurium Besylate, Atropine Sulfate Hydrate, Atropine Sulfate, Azathioprine, Azelastine Hydrochloride, Azithromycin, Azoxystrobin, Aztreonam, Bacampicillin Hydrochloride, Bacitracin Zinc, Bacitracin, Baclofen, Baicalein, Baicalin, Bambuterol Hydrochloride, Bamethan Sulfate, Beclomethasone Propionate, Benserazide Hydrochloride, Benzalkonium Chloride, Benzbromarone, Benzethonium Chloride, Benzoic Acid, Benzydamine Hydrochloride, Benzyl Alcohol, Berberine Chloride Hydrate, Berberine Chloride, Berberine Tannate, Betahistine Mesilate, Betaine, Betamethasone Dipropionate, Betamethasone Sodium Phosphate, Betamethasone Valerate, Betamethasone, Betaxolol Hydrochloride, Bethanechol Chloride, Bezafibrate, α-BHC, β-BHC, γ-BHC, δ-BHC, Bifenthrin, Bifonazole, Bilirubin, Biperiden Hydrochloride, Bisacodyl, Bisdemethoxycurcumin, Bisdemethoxycurcumin, Bisoprolol Fumarate, Bleomycin A2 Hydrochloride, Bornyl Acetate, Bromazepam, Bromhexine Hydrochloride, Bromocriptine Mesilate, 8-Bromotheophylline, Bufalin, Bumetanide, Buspirone Hydrochloride, Busulfan, Butyl Paraoxybenzoate, Cadusafos, Caffeic Acid, Caffeine, Calcitriol, Calcium Folinate, Calcium Gluconate Hydrate, Calcium p-Aminosalicylate Hydrate, Calcium Pantothenate, Calcium Polystyrene Sulfonate, d-Camphor, dl-Camphor, Candesartan Cilexitil, Canthoside D, Capreomycin Sulfate, Capsaicin, Capsaicin, Captan, Captopril, Carbamazepine, Carbazochrome Sodium Sulfonate Hydrate, L-Carbocisteine, Carboplatin, Cardamonin, Carisoprodol, Carmofur, Carteolol Hydrochloride, Carumonam Sodium, Carvedilol, Catalpol, Cathinone, Cefaclor, Cefadroxil, Cefalexin, Cefaloglycin, Cefalotin Sodium, Cefamandole Nafate, Cefamandole, Cefapirin Sodium, Cefatrizine Propylene Glycol, Cefazolin, Cefbuperazone Sodium, Cefcapene Pivoxil Hydrochloride, Cefdinir, Cefditoren Pivoxil, Cefepime Hydrochloride, Cefixime, Cefmenoxime Hydrochloride, Cefmetazole Sodium, Cefmetazole, Cefminox Sodium, Cefodizime Sodium, Cefonicid Sodium, Cefoperazone, Cefotaxime Sodium, Cefotetan, Cefotiam Hexetil Hydrochloride, Cefotiam Hydrochloride, Cefoxitin, Cefpiramide, Cefpirome Sulfate, Cefpodoxime Proxetil, Cefprozil(E) Isomer, Cefprozil(Z) Isomer, Cefradine, Cefroxadine, Cefsulodin Sodium, Ceftazidime, Cefteram Pivoxil Mesitylene Sulfonate, Ceftibuten Hydrochloride, Ceftizoxime Sodium, Ceftizoxime, Ceftriaxone Sodium, Cefuroxime Axetil, Cefuroxime Sodium, Cellacefate, Cetirizine Dihydrochloride, Cetraxate Hydrochloride, Chenodeoxycholic Acid, Chinomethionat, Chlorambucil, Chloramphenicol Palmitate, Chloramphenicol Sodium Succinate, Chloramphenicol, Chlordiazepoxide Hydrochloride, Chlordiazepoxide, Chlorfenapyr, Chlormadinone Acetate, 4-Chlorobenzenesulfoneamide, Chlorogenic Acid, 4-Chlorophenol, Chlorothalonil, Chlorphenesin Carbamate, Chlorpheniramine Maleate, d-Chlorpheniramine Maleate, Chlorpromazine Hydrochloride, Chlorpropamide, Chlorpyrifos, Chlorzoxazone, Cholecalciferol, Chorionic Gonadotropin, Chrysophanol, Ciclacillin, Cilazapril Hydrate, Cilostazol, Cimetidine Hydrochloride, Cimetidine, Cinchonidine, Cinchonine, Cineol, Cinnamic Acid, Cinnarizine, Cinobufagin, Ciprofloxacin Hydrochloride Hydrate, Cisplatin, Citric Acid Monohydrate, L-Citrulline, Clarithromycin, Clavolanic Acid, Clebopride Malate, Clenbuterol Hydrochloride, Clindamycin Hydrochloride, Clindamycin Phosphate, Clinofibrate, Clobetasol Propionate, Clofibrate, Clomifene Citrate, Clomipramine Hydrochloride, Clonazepam, Cloperastine Hydrochloride, Clopidogrel Bisulfate, Clotrimazole, Cloxacillin Sodium, Cocaine Hydrochloride, Codeine Phosphate Hydrate, Codeine Phosphate, Colchicine, Colistin Sodium Methanesulfonate, Coptisine, Cortisone Acetate, Croconazole Hydrochloride, Curcumin, Cyanamide, Cyanocobalamin, Cyanoguanidine, Cyclandelate, Cyclopentolate Hydrochloride, Cyclophosphamide Hydrate, Cyclophosphamide, Cycloserine, Cypermethrin, Cyprodinil, Cyproterone Acetate, Cysteine Hydrochloride, L-Cystine, Cytarabine, Dactinomycin, Daidzin, Dantrolene Sodium Hydrate, Dapsone, Daunorubicin Hydrochloride, pp'-DDD, pp'-DDE, op'-DDT, pp'-DDT, Decursin, Decursinol, Deferoxamine Mesilate, Demethoxycurcumin, Deslanoside, Desoximetasone, Desoxycorticosterone Acetate, Dexamethasone Phosphate, Dexamethasone, Dextromethorphan Hydrobromide, Diacetylated Monoglycerides, Diazepam, Dibekacin Sulfate, Dibucaine Hydrochloride, Dichlorodiamino Cyclohexane Platinum, Diclofenac Sodium, Diclofenamide, Dicloxacillin Sodium, Dicyclomine Hydrochloride, Dieldrin, Diethanolamine Fusidate, Diethylcarbamazine Citrate, Diethylene Glycol, Diflucortolone Valerate, Digitoxin, Digoxin, Dihydrocodeine Phosphate, Dihydroergotamine Mesilate, Dilazep Hydrochloride, Diltiazem Hydrochloride, Dimenhydrinate, Dimercaprol, Dinoprostone, Diosmin, Dioxane, Diphenhydramine Hydrochloride, Dipyridamole, Dirithromycin, Disopyramide, Disulfiram, Dobutamine Hydrochloride, Domperidone Maleate, Domperidone, Dopamine Hydrochloride, Doxapram Hydrochloride Hydrate, Doxazosin Mesilate, Doxorubicin Hydrochloride, Doxycycline, Droperidole, Dydrogesterone, Ebastine, Edrophonium Chloride, Elcatonin, Emetine Hydrochloride, Emodin, Enalapril Maleate, Endosulfan, Endotoxin, Endrin, Enflurane, Enoxacin Hydrate, Enviomycin Sulfate, Ephedrine Hydrochloride, Ephedrine Hydrochloride, Ephedrine Sulfate, Epinephrine Tartrate, Epirubicin Hydrochloride, Ergocalciferol, Ergometrine Maleate, Ergotamine Tartrate, Erythromycin Estolate, Erythromycin Ethylsuccinate, Erythromycin Lactobionate, Erythromycin Oxime, Erythromycin Stearate, Erythromycin, Esomeprazole Strontium, Estradiol Benzoate, Estradiol Valerate, Estradiol, Estragole, Estriol, Estrone, Etacrynic Acid, Ethambutol Hydrochloride, Ethanol, Ethenzamide, Ethinylestradiol, Ethionamide, Ethosuximide, Ethyl Aminobenzoate, Ethyl Paraoxybenzoate, Ethylene Glycol, Ethylvanillin, Etizolam, Etodolac, Etoposide, Eugenol, Evodiamine, Famotidine, Felodipine, Fenarimol, Fenofibrate, Fenoprofen Calcium Dihydrate, Fenoprofen Calcium, Fenoterol Hydrobromide, Fenpropathrin, Fentanyl Citrate, Fenticonazole Nitrate, Ferrous Fumarate, Ferrous Gluconate II, Ferrous Sulfate, Finasteride, Flavin Adenine Dinucleotide Sodium, Flavoxate Hydrochloride, Flomoxef Sodium, Flomoxef Triethylammonium, Flubendazole, Flucloxacillin Sodium, Flucytosine, Fludioxonil, Fludrocortisone Acetate,

Flumequine, Flunarizine Dihydrochloride, Flunitrazepam, Flunitrazepam, Fluocinolone Acetonide, Fluocinonide, 9-Fluorenylmethy Chloroformate, Fluorometholone, Fluoroquinolonic Acid, Fluorouracil, Fluoxetine Hydrochloride, Fluoxymesterone, Fluphenazine Enanthate, Flurazepam Hydrochloride, Flurazepam, Flurbiprofen, Fluticasone Propionate System Suitability Mixture {Mixture of Fluticasone Propionate RS and Fluticasone Propionate Related Substance II, III and IV}, Fluticasone Propionate System Suitability Mixture (Mixture of fluticasone propionate and fluticasone propionate related substance II III and IV), Fluticasone Propionate, Folate, Folic acid, Formononetin, Formoterol Fumarate Hydrate Dihydrate, Formoterol Fumarate Hydrate, Forsythoside A, Fosfomycin Phenethylammonium, Fosfomycin, Fosthiazate, Fructose, Fumaric Acid, Furosemide, Fursultiamine Hydrochloride, Fusidic Acid Hydrate, Fusidic Acid, Gabexate Mesilate, Gallamine Triethiodide, Gardenoside, Geniposide, Gentamicin Sulfate, Gentiopicroside, Germacrone, 6-Gingerol, Ginsenoside Rb1, Ginsenoside Rg1, Gitoxin, Glibenclamide, Gliclazide, Glimepiride, Gliquidone Sulfonamide, Glucose, Glutamic Acid, Glutamine, Glycerin, Glycine, Glycyrrhizic Acid, Gomisin A, Gomisin N, Gramicidin, Griseofulvin, Guaifenesin, Guanethidine Sulfate, Guanine, Haloperidol, Halothane, E-Harpagoside, Heparin Sodium, Hesperidin, Hexaconazole, High Molecular Weight Urokinase, Honokiol, Hydralazine Hydrochloride, Hydrochlorothiazide, Hydrocortisone Acetate, Hydrocortisone Butyrate, Hydrocortisone Sodium Succinate, Hydrocortisone Succinate, Hydrocortisone, Hydrocotarnine Hydrochloride, Hydroxychloroquine Sulfate, 20-Hydroxyecdysone, 5-Hydroxymethyl-2-furaldehyde, Hydroxyprogesterone Caproate, Hydroxypropyl Cellulose, Hymecromone, Hyosine Butylbromide, Hypromellulose Phthalate, Ibuprofen, Icariin, Idarubicin Hydrochloride, Idoxuridine, Ifenprodil Tartrate, Imidazole, Imipenem, Imipramine Hydrochloride, Imperatorin, Imperatorin, Indapamide, Indigocarmine, Indometacin, Inositol, Insulin, Iodine, Iodixanol, Iodomethane, Iohexol, Iopamidol, Iopanoic Acid, Iopromide, Iospropyl Iodide, Iotalamic Acid, Ipratropium Bromide Hydrate, Isepamicin Sulfate, Isoconazole Nitrate, Isoflurane, Isoimperatorin, L-Isoleucine, Isomalt, Isoniazid, Isoproterenol Hydrochloride, Isosorbide Dinitrate, Isosorbide, Isotretinoin, Isradipine, Josamycin, Kallidinogenase, Kanamycin Sulfate, Ketamine Hydrochloride, Ketoconazole, Ketoprofen, Ketorolac Tromethamine, Ketotifen Fumarate, Kresoxim-methyl, Lacidipine, Lactitol Hydrate, Lactose Hydrate, Lactose, Lactulose, Lamivudine, Lansoprazole, Latamoxef Ammonium, Latamoxef Sodium, Leonurine, Letrozole, L-Leucine, Leucomycin A5, Leucovorin Calcium, Levodopa, Levodropropizine, Levothyroxine Sodium Hydrate, Lidocaine, Lincomycin Hydrochloride Hydrate, Linderane, Liothyronine Sodium, Liquiritin, Liquirtigenin, Lisinopril Hydrate, Lithium Carbonate, Lithocholic Acid, Loganin, Loperamide Hydrochloride, Loracarbef, Lorazepam, Losartan Potassium, Lovastatin, Loxoprofen Sodium Hydrate, Loxoprofen, Lysine Hydrochloride, Magnesium Aspartate Hydrate, Magnolol, Maltitol, Maltose Hydrate, Mangiferin, D-Mannitol, Maprotiline Hydrochloride, Matrine, Mebendazole, Mebeverin Hydrochloride, Meclizine Hydrochloride, Meclocycline, Meclofenoxate Hydrochloride, Mecobalamin, Medroxyprogesterone Acetate, Mefenamic Acid, Megestrol Acetate, Melamine, Meloxicam, Melphalan, l-Menthol, Mepivacaine Hydrochloride, Meprobamate, Mequitazine, Mercaptopurine, Meropenem, Mesalazine, Mestranol, Metenolone Enanthate, Metformin Hydrochloride, Methacycline Hydrochloride, Methamphetamine Hydrochloride, Methanol, DL-Methionine, Methocarbamol, Methotrexate, Methoxsalen, Methoxychlor, Methoxyethanol, Methyl Iodide, Methyl Paraoxybenzomate, Methyl Pentachlorophenyl Sulfide, Methylcarbamate, Methyldopa, dl-Methylephedrine Hydrochloride, Methylergometrine Maleate, Methylphenidate Hydrochloride, Methylprednisolone Sodium Succinate, Methylprednisolone, Methyltestosterone, Metiram, Metoclopramide Hydrochloride, Metoclopramide, Metronidazole, Mexiletine Hydrochloride, Miconazole Nitrate, Micronomicin Sulfate, Midazolam, Midecamycin Acetate, Midecamycin, Minocycline Hydrochloride Hydrate, Mitomycin C, Mometasone Furoate, Morphine Hydrochloride Hydrate, Morphine Sulfate Hydrate, Morronisede, Mosapride Citrate, Mupirocin Lithium, Mupirocin, Myclobutanil, Nabumetone, Nalidixic Acid, Naloxone Hydrochloride, Naphazoline Nitrate, Napropamide, Naproxen Sodium, Naproxen, Naringin, Neomycin Sulfate, Neostigmine Bromide, Neostigmine Methylsulfate, Netilmicin Sulfate, Nicardipine Hydrochloride, Nicergoline, Nicorandil, Nicotinic acid amide, Nicotinic Acid, Nifedipine, Nifuroxazide, Nimodipine, Nitrazepam, Nitrendipine, Nitroglycerin, Nizatidine, Nodakenin, Nordazepam, Norepinephrine Tartrate, Norethisterone Acetate, Norethisterone, Norfloxacin, Norgestrel, Nortriptyline Hydrochloride, Noscapine Hydrochloride Hydrate, Noscapine, Nystatin, Ofloxacin, Oleanolic Acid, Omeprazole, Ondansetron Hydrochloride Hydrate, Ondansetron Hydrochloride, Orciprenaline Hydrochloride, Orciprenaline Sulfate, Orphenadrine Hydrochloride, Oxaliplatin, Oxapium Iodide, Oxaprozin, Oxethazaine, Oxprenolol Hydrochloride, Oxybuprocaine Hydrochloride, Oxycodone Hydrochloride Hydrate, Oxymatrine, Oxymetazoline Hydrochloride, Oxymetholone, Oxypeucedanin, Oxytetracycline, Oxytocin, Paclitaxel, Paeoniflorin, Paeonol, Palmatine, Palmitic Acid, Panipenem, Papaverine Hydrochloride, Paroxetine Hydrochloride Hydrate, Penbutolol Sulfate, Pendimethalin, Penicillin G Potassium, Penicillin G Sodium, Pentachloroaniline, Pentazocine, Pentobarbital, Pentoxifylline, Pentoxyverine Citrate, Perphenazine Maleate, Perphenazine, Pethidine Hydrochloride, Phenobarbital, Phenol, Phenolsulfonphthalein, L-Phenylalanine, Phenylephrine Hydrochloride, Phenytoin, Phloroglucinol, Physcion, Phytonadione, Pimaricin, Pimozide, Pindolol, Pinoresinol Diglucoside, Pipemidic Acid Hydrate, Piperacillin, Piperazine Citrate, piperidine hydrochloride, Piperine, Piracetam, Pirarubicin, Pirenzepine Hydrochloride Hydrate, Piroxicam, Pivampicillin, Pivmecillinam Hydrochloride, Polydimethylsiloxane, Polymyxin B Sulfate, Polysorbate 80, Poncirin, Potassium Gluconate, Potassium Guaiacolsulfonate, Potassium Iodide, Potassium Sucrose Octasulfate, Povidone, Pralidoxime Chloride, Pranoprofen, Pravastatin 113.3-Tetramethylbutylammonium, Pravastatin Sodium, Prednisolone Acetate, Prednisolone Succinate, Prednisolone, Primidone, Probenecid, Procainamide Hydrochloride, Procaine Hydrochloride, Procaterol Hydrochloride Hydrate, Prochloraz, Prochlorperazine Maleate, Procymidone, Progesterone, Proglumide, Promethazine Hydrochloride, Propafenone Hydrochloride, Propafenone, 2-Propanol, Propineb, Propofol, Propranolol Hydrochloride, Propyl Paraoxybenzoate, Propylthiouracil, Protamine Sulfate, Protirelin, Pseudoephedrine Hydrochloride, Puerarin, Pyrantel Pamoate, Pyrazinamide, Pyridostigmine Bromide, Pyridoxine Hydrochloride, Pyrimethanil, Pyrvinium Pamoate, Quercetin Dihydrate, Quinine Sulfate Hydrate, Quintozene, Ramipril, Ranitidine Hydrochloride, Redibufogenin, Repaglinide, Reserpine, Retinol Acetate, Retinol Palmitate, Rhein, Riboflavin Butyrate, Riboflavin Sodium Phosphate, Riboflavin, Ribostamycin Sulfate, Rifabutin, Rifampicin, Rifamycin SV, Rifaximin, Risperidone, Rokitamycin, Roxithromycin, Rutecarpin, Rutin, Saccharated Pepsin, Saccharin Sodium Hydrate, Saccharin, Saikosaponin A, Saikosaponin D, Salbutamol Sulfate, Salicylic Acid, Salvianolic Acid b, Sarpogrelate Hydrochloride,

Sarsasapogenin, Schisandrin, Scopolamine Butylbromide, Scopolamine Butylbromide, Selegiline Hydrochloride, Sennoside A, Sennoside B, Silver Sulfadiazine, Simethicone, Simvastatin, Sisomicin Sulfate, β-Sitosterol, Sodium Citrate Hydrate, Sodium Cromoglicate, Sodium Hyaluronate, Sodium Picosulfate Hydrate, Sodium Polystyrene Sulfonate, Sodium Salicylate, Sodium Valproate, D-Sorbitol, Spectinomycin Hydrochloride, Spiramycin, Spironolactone, Stannous Fluoride, Stearic Acid, Streptomycin Sulfate, Strychnine Nitrate, Sulbactam Sodium, Sulbactam, Sulbenicillin Sodium, Sulfamethizole, Sulfamethoxazole, Sulfasalazine, Sulfinpyrazone, Sulpiride, Sultamicillin Tosylate, Suxamethonium Chloride Hydrate, Suxamethonium Chloride, Swertiamarin, Tamoxifen Citrate, Tamsulosin hydrochloride, Tansinone IIA, Tebuconazole, Tebufenpyrad, Tegafur, Teicoplanin, Telmisartan, Temazepam, Tenoxicam, Terazosin Hydrochloride, Terbufos, Terbutaline Sulfate, Terconazole, Testosterone Enanthate, Testosterone Propionate, Tetracycline Hydrochloride, Tetrahydropalmatine, 2,3,5,4'-Tetrahydroxystilben- 2-O-β-D-glucoside, Tetrahydrozoline Hydrochloride, Theophylline, Thiamazole, Thiamine Hydrochloride, Thiamphenicol, Thiamylal Sodium, Thiamylal, Thianthol, Thifluzamide, Thiopental, Thioridazine Hydrochloride, Thiram, L-Threonine, Thrombin, Thymol, Tiapride Hydrochloride, Tiapride N-Oxide, Tiaprofenic Acid, Ticarcillin Sodium, Ticlopidine Hydrochloride, Timolol Maleate, Tinidazole, Tipepidine Hibenzate, Tipepidine Hibenzate, Tobramycin, Tocopherol Acetate, Tocopherol Calcium Succinate, Tocopherol Succinate, Tocopherol, Tofisopam, Tolazoline Hydrochloride, Tolbutamide, Tolfenamic Acid, Tolnaftate, Tolylfluanid, Torsemide, Tramadol Hydrochloride, Tranexamic Acid, Trapidil, Triadimefon, Triadimenol, Triamcinolone Acetonide, Triamcinolone, Triamterene, Trichlormethiazide, Triclosan, Triflumizole, Triflusal, Trihexyphenidyl Hydrochloride, Trimebutine Maleate, Trimetazidine Hydrochloride, Trimetoquinol Hydrochloride Hydrate, L-Tryptophan, Tubocurarine Chloride Hydrochloride Hydrate, Tulobuterol Hydrochloride, Ubidecarenone, Uracylarabinoside, Ursolic Acid, L-Valine, Valsartan, Vancomycin Hydrochloride, Verapamil Hydrochloride, Vigabatrin, Vinblastine Sulfate, Vincristine Sulfate, Vinorelbine Tartrate, 5-vinyl-2-pyrrolidone, Voglibose, Woogonin, Xanthantanoic Acid, Xanthon, Xylitol, Zaltoprofen, Zolpidem Tartrate

Reference Medicinal Plant Materials (RMPM)
Achyranthes Root RMPM, Acori Graminei Rhizoma RMPM, Adenophorae Radix RMPM, Agastachis Herba RMPM, Akebia Stem RMPM, Alisma Rhizome RMPM, Amomum Fruit RMPM, Anemarrhena Rhizome RMPM, Anethi Fructus RMPM, Angelica Dahurica Root RMPM, Angelica Gigas Root RMPM, Apricot Kernel RMPM, Aralia Continentalis Root RMPM, Areca RMPM, Arisaema Rhizome RMPM, Artemisiae Capillaris Herba RMPM, Asiasarum Root and Rhizome RMPM, Asparagus Tuber RMPM, Astragalus Root RMPM, Atractylodes Rhizome RMPM, Atractylodes Rhizome White RMPM, Aurantii Fructus Immaturus RMPM, Brassicae Semen RMPM, Bupleurum Root RMPM, Chelidonii Herba RMPM, Cinnamon Bark RMPM, Citrii Unshiu Immature Peel RMPM, Citrus Unshiu Peel RMPM, Cnidium Rhizome RMPM, Codonopsis Pilosula Root RMPM, Common Cnidium Fruit RMPM, Coptis Rhizome RMPM, Cornus Fruit RMPM, Curcuma Longa Rhizome RMPM, Curcuma Root RMPM, Cynanchi Wilfordii Radix RMPM, Cyperus Rhizome RMPM, Dioscorea Rhizome RMPM, Dipsaci Radix RMPM, Epimedium Herb RMPM, Eucommia Bark RMPM, Fennel RMPM Fritillaria Thunbergii Bulb RMPM, Gardenia Fruit RMPM, Gastrodia Rhizome RMPM, Gentian Root and Rhizome RMPM, Glehnia Root RMPM, Houttuyniae Herba RMPM, Leonurus Herb RMPM, Licorice RMPM, Ligustici Tenuissimi Rhizoma Et Radix RMPM, Liriope Tuber RMPM, Lithospermum Root RMPM, Lonicera Flower RMPM, Lonicera Leaf and Stem RMPM, Lycium Fruit RMPM, Moutan Root Bark RMPM, Mulberry Root Bark RMPM, Ostericum Root RMPM Peach Kernel RMPM, Peony Root RMPM, Perilla Leaf RMPM, Peucedani Radix RMPM, Pinellia Tuber RMPM, Plantago Seed RMPM, Platycodon Root RMPM, Polygala Root RMPM, Polygonatum Rhizome RMPM, Polygonum Multiflorum Root RMPM, Poncirus Immature Fruit RMPM, Pueraria Root RMPM, Pulvis Aconiti Tuberis Purificatum RMPM, Raphanus Seed RMPM, Rehmannia Root RMPM, Rubus Fruit RMPM, Safflower RMPM, Saposhnikovia Root RMPM, Schisandra Fruit RMPM, Schizonepeta Spike RMPM, Scrophularia Root RMPM, Scutellaria Root RMPM, Sinomenium Stem and Rhizome RMPM, Smilacis Rhizoma RMPM, Sophora Root RMPM, Sparganium Rhizome RMPM, Zizyphus Seed RMPM

Reference Standards of Related Substances
Acenaphthene, 3-(Acetylthio)-2-methylpropanoic acid, β-Alanine, Alfuzosin related substance I {N-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide Hydrochloride}, (E)-4-Amino-2-ethylidenebutyric acid hydrochloride, 2-Amino-4-chlorophenol, 2-Amino-5-chlorobenzophenone, p-Aminobenzoyl glutamic acid, p-Aminobenzoylglutamic Acid, 2-Aminobutanol, N-aminohexamethyleneimine, 3-Aminopent-4-ene-1,1-dicarboxylic acid, Amitriptyline {5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ol}, Amlodipine Besylate related substance I {3-Ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate}, L-Arabinitol {l-arabitol 1,2,3,4,5-pentanephenol}, Atorvastatin Related Substance I {(3R,5R)-7-[3-(Phenylcarbamoyl)5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid calcium salt)}, Benzydamine Hydrochloride related substance I {3-(Dimethylamino)propyl 2-benzylaminobenzoate Hydrochloride}, Benzydamine Hydrochloride related substance II {3-(1,5-Dibenzyl-1H-indazole-3-yl)oxypropyldimethylamine}, Benzydamine Hydrochloride related substance III {1-Benzyl-1H-indazole-3-ol}, Captopril Disulfide, Carvedilol related substance I {(2RS-1-Benzyl[2-(2-methoxyphenoxy)ethyl]amino-3-(9H-carbazol-4-yloxy)propan-2-ol)}, Carvedilol related substance II {1-[[9-[2-Hydrocy-3-[[2-(2-methoxyphenoxy)ethyl]-amino]propyl]-9H-carbazole-4-yl]oxy]-3-[[2-(2-methoxy-phenoxy)ethyl]amino]propane-2-ol}, Carvedilol related substance III {11'-[[2-(2-Methoxyphenoxy)ethyl]nitrile]bis[3-(9H-carbazole-4-yloxy)propane-2-ol]}, Cefaclor Delta-3 Isomer, Ceftazidime high molecular polymer, Cetirizine related substance I {(RS)-1-[(4-Chlorophenyl)phenylmethyl]piperazine}, Chenodeoxycholic Acid, 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide, 3-Chloro-2-methylaniline, 2-Chloro-4-N-furfurylamino-5-sulfamoylbenzoic acid, 4-Chlorobenzenesulfoneamide, 2-Chlorobenzoic acid, (2-Chlorophenyl)-diphenylmethanol, Cilazapril related substance I {1,1-dimethylethyl(1S,9S)-9-[[(S)-1-(ethoxycarbonyl)-3-phenypropyl]amino]-10-oxo-ocatahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-carboxylate}, Cilazapril related substance II {(1S,9S)-9-[[(S)-1-carboxy-3-phenylpropyl]amino]-10-oxo-octahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid}, Cilazapril related substance III {ethyl(1S,9S)-9-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxo-octahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-Carboxylate}, Cilazapril related substance

IV {(1S,9S)-9-[[(S)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxo-octahydro-6H-pyridizano[1,2-a][1,2]diazepine-1-carboxylic acid}, Cinchonidine, Cinchonine, Ciprofloxacin Ethylenediamine derivative, Clenbuterol Hydrochloride related substance I {1-(4-Amino-3,5-dichlorophenyl)-2-[(1,1-dimethylethyl)amino]ethenone (clenbuterol-ketone)}, Clomiphene related substance I {(E,Z)-2-[4-(1,2-Diphenylethenyl)phenoxy]-N,N-diethylethaneamine hydrochloride}, Clopidogrel Related Substance I {(+)-(S)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid}, Clopidogrel related substance II {Methyl (±)-(o-Chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate hydrochloride}, Clopidogrel related substance III {Methyl(-)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate hydrogen sulfate}, Cyclobenzaprine Hydrochloride, Dermatan Sulfate, Dibenzosuberone {10,11-dihydro-5H-Dibenzo[a,d]cycloheptene-5-one}, Diclofenac Sodium, Diethylene Glycol, 3,4-Dihydro-6-hydrocy-2(1H)-quinoline, Diosmine related substance I {1-(3-Hydroxy-4-methoxyphenylethanone(acetoisovanillone)}, Diosmine related substance II {(2S)-7-[[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-Glaucopyranosyl]oxy]-5-hydrocy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-l-benzopyran-4-one(hesperidin)}, Diosmine related substance III {7-[[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-Glaucopyranosyl]oxy]-5-hydrocy-2-(4-methoxyphenyl)-4H-l-benzopyran-4-one(isorhoifolin)}, Diosmine related substance IV {7-[[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-Glaucopyranosyl]oxy]-5-hydrocy-2-(3-hydrocy-4-methoxyphenyl)-6-iodo-4H-l-benzopyran-4-one(6-iododiosmine)}, Diosmine related substance V {7-[[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-Glaucopyranosyl]oxy]-5-hydrocy-2-(4-methoxyphenyl)-4H-l-benzopyran-4-one(linarin)}, Diosmine related substance VI {5,7-Dehydrocy-2-(3-hydrocy-4-methoxyphenyl)-4H-l-benzopyran-4-one(diosmetin)}, 1,1-Diphenyl-4-piperidino-1-butene hydrochloride, Ebastine related substance I {Diphenylethanol (benzhydrol)}, Ebastine related substance II {1-[4-(1,1-Dimethylethyl)phenyl]ethanol}, Ebastine Related Substance III [4-(Diphenylmethoxy)piperidine], Ebastine Related Substance IV {1-[4-(1,1-Dimethylethyl)phenyl]-4-(4-hydroxypiperidine-1-yl)butan-1-one], Ebastine related substance V {1-[4-(1,1-Demethylpropyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one}, Ebastine related substance VI {1-[4-(1,1-Demethylethyl)phenyl]-4-[cis-4-(diphenylmethoxy)-1-oxydopiperidin-1-yl]butan-1-one}, Ebastine related substance VII {1-[4-(1,1-Demethylethyl)phenyl]-4-[trans-4-(diphenylmethoxy)-1-oxydopiperidin-1-yl]butan-1-one}, Econazole Nitrate, Enalaprilate, 4-Epianhydrotetracycline hydrochloride, Epilactose, Epitiostanol, Erythromycin A iminoether, 2-Ethyl-2-phenylmalonamide, Etodolac Related Substance I, Fenofibrate related substance I {(4-Chlorophenyl)(4-hydroxyphenyl)methanone}, Fenofibrate related substance I {(4-Chlorophenyl)(4-hydroxyphenyl)methanone}, Fenofibrate related substance II {2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid, Fenofibrate related substance III {1-Methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate}, Fenofibrate related substance IV {(3RS)-3-[4-(4-Chlorobenzoyl)phenoxy]butan-2-one}, Fenofibrate Related Substance V {methyl 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoate}, Fenofibrate Related Substance VI {ethyl 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoate}, Fenofibrate Related Substance VII {(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanones}, Fenticonazole Nitrate Related Substance I {(RS)-1-[2-(2,4-Dichlorophenyl)-2-hydroxyethyl]-3-[4-(phenylsulfanyl)benzyl]imidazolium Nitrate}, Flumequine Related Substance I {Ethyl (RS)-9-Fluoro-5-methyl-1-oxo-6,7-dihydro-1H,5H-benzo[i,j]quinolizine-2-carboxylate (Flumequine Ethyl Ester)}, 9α-Fluoro-11β-hydroxy-16β-methyl-3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3-(2'H)-one], 4[4-(Fluoro-11β-hydroxy-16β-methyl-3-oxo-androsta-1,4-diene-17-spiro-2'-(9α)-hydrophenylmethyl)-1-piperidinyl]-1-butanone, Fluoxetine related substance I {N-Methyl-3-phenyl-[(α,α,α-(trifluoro-m-tolyl)oxy)propylamine hydrochloride}, Fluoxetine related substance II {N-Methyl-3-phenylpropylamine}, Fluticasone propionate related substance I {[6α,9α-Difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbonylsulfenic acid}, Fluticasone propionate related substance II {6α,9α-Difluoro-11β-hydroxy-16α-methyl-2',3,4'-trioxo-17α-spiro(androsta-1,4-diene-17,5'-(1,3)oxathiolane)}, Fluticasone propionate related substance III {S-Fluorinemethyl 17α-acetyloxy-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioate}, Fluticasone propionate related substance IV {S-Methyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17β-carbothioate}, Fluticasone propionate related substance V {6α,9α-Difluoro-11β,17α-dihydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carboxylic acid 6α,9α-difluoro-17β-(fluoromethylthio)carbonyl-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-dien-17α-yl ester}, Formoterol Fumarate Hydrate, Galactose, Gentamycin B, Gitoxin, Glactitol {Dulcitol}, Gliclazide related substance I [2-Nitroso-octahydrocyclopenta[c]pyrrole], Gliclazide related substance II [1-(Hexahydrocyclopenta[c] pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea], Guanine, Hydrochlorothiazide related substance I {4-Amino-6-chloro-1,3-benzenedisulfonamide}, 1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol, N-(2-Hydroxyethyl)isonicotinamide nitric ester, 5-Hydroxymethyl-2-furaldehyde, 9-Hydroxypropantheline Bromide, Hyoscine Hydrobromide Imipramine Hydrochloride, Iodixanol related substance I {5-[Acetyl[3-[[3,5-bis[[2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]-2-hydroxypropyl] amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide}, Iodixanol related substance II {5-[Acetyl(2-hydroxy-3-methoxypropyl)amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide}, Iodixanol related substance III RS {5-(Acetylamino)-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide}, Iodixanol related substance IV RS {2-[[Acetyl[3,5-bis[[2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-dicarboxamide}, Iodixanol related substance V RS {4-Acetyl-2-[[acetyl[3,5-bis[[2,3-dihydroxypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dihydroxypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-dicarboxamide}, Iodixanol related substance VI {2-[[Acetyl[35-bis[[23-dihydroxypropyl) amino]carbonyl]-246-triiodophenyl]amino]methyl]-N,N'-bis(23-dihydroxypropyl)-23-dihydro-57-diiodo-4H-14-benzoxazine-68-dicarboxamide}, Iodixanol Related Substance VII, Iohexol related substance I {5-(Amino)-N,N-bis(2,3-dihydroxypropyl)-2,4,6-triiod-1,3-benzene-dicarboxamide)}, Iohexol related substance II {5-(Acetylamino)-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzene-dicarboxamide}, Iohexol related substance III {N,N'-Bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzene-dicarboxamide}, Iohexol Related Substance IV, Iopromide related substance I {[5-(Acetylamino)-N,N'-bis(2,3-dihydroxypropyl-2,4,6-triiodo-N-methyl-1.3-benzene-dicarboxamide}, Iopromide Related Substance II, Isobutylpiperidone, Isonicotinic acid amide, Isopromethazine Hydrochloride, Isradipine related substance I {Isopropylmethyl 4-(4-benzofurzanyl)-2,6-dimethyl-3,5-pyridinedicarboxylate}, Lacidipine related substance I {Diethyl(E)-

4-{2-[2-tert-butoxycarbonyl)vinyl]phenyl}-2,6-dimethylpyridine-3,5-dicarboxylate}, Lactose Anhydride, Lamivudine resolution mixture I, Lamivudine resolution mixture II, Lansoprazole related substance I {2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole}, Letrozole related substance I {4,4'-(1H-1,3,4-Triazol-1-ylmethylene)dibenzonitrile}, Levodropropizine related substance I {1-Phenylpiperazine}, Levodropropizine related substance II {(2RS)-Oxiran-2-yl]methanol (glycidol)}, Lithocholic Acid, Lovastatin related substance I {Dihydrolovastatin}, Maleic acid, Medroxyprogesterone acetate Related Substance I, Meloxicam related substance I {Ethyl-4-hydrocy-2-methyl-2H-1,2-benzothiazine-3-Carboxylate-1,1-deoxide}, Meloxicam related substance II {5-Methylthiazole-2-ylamine}, Meloxicam related substance III {4-Hydrocy-2-methyl-N-ethyl-N'-(5-methyl-13-thiazole-2-yl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide}, Meprobamate, Mesalazine related substance I {2-Aminophenol), Mesalazine related substance II {4-Aminophenol), Mesalazine related substance III {3-Aminophenol), Mesalazine related substance IV {Aniline), Mesalazine related substance V {3-Aminobezoic acid), Mesalazine related substance VI {2,5-Dihydroxybezoic acid), Mesalazine related substance VII {Salicylic acid), Methotrexate Related Substance I {(S)-2-{4-[(2,4-deaminopteridine-6-yl)methylamino]benzamido}pentanedioate}, Methotrexate Related Substance II {(S)-2-(4-{[(2-amino-4-oxo-1,4-dehydropteridine-6-yl)methyl](methyl)amino}benzamido)pentanedioate}, Methotrexate Related Substance III {4-{[(2,4-deaminopteridine-6-yl)methyl]methylamino}benzoic acid 1/2hydrochloride}, 1-Methyl-4-(2-benzoylhydrazino)azepan hydrochloride, 1-Methyl-4-(2-benzoylhydrazino)azepan hydrochloride, 2-Methyl-5-nitroimidazole 1-Methylazepan-4-one hydrochloride, 3-O-Methylmethyldopa, Methylphenidate Hydrochloride erythro isomer, N-Methylpyrrolidine, Nabumetone related substance I [1-(6-Methoxy-2-naphthyl)-but-1-ene-3-one], Nifedipine nitrophenylpyridine analog {Dimethyl4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-di-Carboxylate}, Nifedipine nitrosophenylpyridine analog {Dimethyl4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-diCarboxylate}, Nifuroxazide related substance I {4-Hydroxybenzohydrazide}, Nifuroxazide related substance II {Methyl p-hydroxybenzoate}, Nifuroxazide related substance III {(5-Nitrofuran-2-yl)methylenediacetate}, Nifuroxazide related substance IV {1,2-Bis[(5-nitrofuran-2-yl)methylen]diazan(5-nitrofurfuralazine)}, Nimodipine related substance I {2-Methoxyethyl-methylethyl-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate}, Nitrilotriacetic Acid, Ondansetron Related Substance I (1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one), Ondansetron Related Substance II (1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one), Ondansetron Related Substance III {3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one}, Ondansetron Related Substance IV {6,6'-Methylene-bis-[(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)-methyl]-4H-carbazol-4-one}, Orphenadrine related substance I {(RS)-N,N-Dimethyl-2-[(3-methylphenyl)phenylmethoxy]ethanamine (meta-methylbenzyl isomer)}, Oversulfated Chondroitin Sulfate, Oxaliplatin Related Substance I (oxalic acid), Oxaliplatin related substance II {(SP-4-2-)-Diaqua[(1R,2R)-cyclohexane-1,2-diamine-κN,κN']platinum (diaquodiaminocyclohexane platinum)}, Oxaliplatin Related Substance III {(OC-6-33)-[(1R,2R)-cyclohexane-1,2-diamine-κN,κN'][ethanedioato(2-)-κO1,κO2]dihydroxyplatinum}, Oxaliplatin Related Substance IV {(SP-4-2)-[(1S,2S)-Cyclohexane-1,2-diamine-κN,κN'][ethanediato(2-)-κO1,κO2]platinum}, Oxaliplatin related substance V {(SP-4-2)-De-μ-oxo-bis[(1R,2R)-cyclohexane-1,2-diamine-κN,κN']deplatinum}, Paclitaxel related substance II {10-Deacetyl-7-epi-paclitaxel}, Paclitaxel related substance II {10-Deacetyl-7-epi-paclitaxel}, Paclitaxel related substances I {Cephalomannine}, Paroxetine related substance I {(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl)piperidine((+)-trans-Paroxetine)}, Paroxetine related substance II {(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl)piperidine}, Paroxetine related substance III {(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-phenylpiperidine, Desfluoroparoxetine}, α-Phenyl-2-piperidineacetic acid hydrochloride, Phenytoin related substance I {Diphenylglycine}, Phenytoin related substance II {Diphenylhydantoic acid}, Phloroglucinol related substance I {Pyrogallol}, Phloroglucinol related substance II {Phlorogluc ide}, Piperidine Hydrochloride, Piracetam related substance I {2-Pyrrolidone}, Primidone related substance I {2-Ethyl-2-phenylmalonamide}, Primidone related substance II {2-Phenyl butyramide}, Propofol related substance I {2,0-Bis(1-methylethyl)-1,4-benzoquinol}, Propofol related substance II {2-(1-Methylethoxy)-1,3-bis(1-methylethyl)benzene}, Propofol related substance III {3,3',5,5'-Tetrakis(1-methylethyl)biphenyl-4,4'-diol}, Ramipril related substance I {[2S,3aS,6aS)-1-[(S)2-[[(S)1-(Methoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid}, Ramipril related substance II {[(2S,3aS,6aS)-1-[(S)2-[[(S)1-(Methylethoxy)carbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid}, Ramipril related substance III {[(2S,3aS,6aS)-1-[(S)2-[[(S)1-(Ethoxycarbonyl)-3-cyclohexylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid}, Ramipril related substance IV {[Ethyl(2S)2-(3S,5aS,8aS,9aS)-3-methyl-14-deoxodecahydro-1H-cyclopenta[e]pyrrolo[12-a]pyrazin-2-yl]-4-phenylbutanoate}, Ranitidine related substance I, Ranitidine related substance II, Ranitidine related substance III, Repaglinide related substance I {(S)-3-Methyl-1-[2-(1-piperidinyl)phenyl]butylamine N-acetyl-L-glutamate salt}, Repaglinide related substance II {3-Ethoxy-4-ethoxycarbonylphenylacetate}, Repaglinide related substance III RS {(S)-2-Ethoxy-4-[2-[[2-phenyl-1-[2-(1-piperidinyl)phenyl]ethyl]amino]-2-oxoethyl]benzoate}, Rifamycin B, Sorbitol, Telmisartan related substance I {1,7'-Dimethyl-2'-propyl-1H,3'H-2,5'-bibenzo[d]imidazole}, Telmisartan related substance II {4'-[(1,7'-Dimethyl-2'-propyl-1H,1'H-2,5'-bibenzo[d]imidazol-1'-yl)methyl]biphenyl-2-carboxylic acid}, Terazosin related substance I {1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine dihydrochloride}, Terazosin related substance II {1-(4-Hydrooxy-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)carbonyl]piperazine}, Terazosin related substance III {1,4-bis(4-Amino-6,7-dimethoxy-2-quinazolinyl)piperazine, dihydrochloride}, Terbutaline related substance I {3,5-Dihydroxy-ω-t-butylaminoacetophenone sulfate}, 1-(Tetrahydro-2-furanyl)piperazine, Tetrahydrofuran-2-carboxylic acid, 1H-tetrazol-5-thiol, Tiapride Related Substance I {N,N-Diethylethane-1,2-diamine}, Tiaprofenic acid related substance I {(2RS)-2-(5-Benzoylthiopen-3-yl)propanoic acid}, Tiaprofenic acid related substance II {(5-Ethylthiophen-2-yl)phenylmethanones}, Tiaprofenic acid related substance III {1-(5-Benzoylthiophen-2-yl)ethanone}, Tolfenamic acid related substance I {2-Chlorobenzoic acid}, Tolfenamic acid related substance II {2-Chloro-2-methylaniline}, Torsemide related substance I {4-[(3-Methylphenyl)amino]-3-pyridinesulfonamide}, Torsemide related substance II {N-[(n-Butylamino)carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfoneamide}, Torsemide related substance III {N-[Ethylamino)carbonyl]-4-[(3-

methylphenyl)amino]-3-pyridinesulfoneamide}, Tramadol Related Substance I {(2RS)-2-[(Dimethylamino)methyl]cyclohexanone}, Tramadol Related Substance II {(1RS,2SR)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol}, Tretinoin, Tribenoside related substance I {3,5,6-Tri-O-benzyl-1,2-O-(1-methyl-ethylidene)-α-D-glucofuranose}, Tribenoside related substance II {Benzaldehyde}, Tribenoside related substance III {Dibenzylether}, Tribenoside related substance IV {3,5-Di-O-benzyl-1,2-O-(1-methylethylidene)-α-D-glaucofuranose}, Triflusal related substance I {2-Acetoxyterephthalic acid}, Triflusal related substance II {4-(Trifluoromethyl)salicylic acid}, Trihexyphenidyl related substance I {1-Phenyl-3-(piperidin-1-yl)propan-1-one}, Urea, Valsartan related substance I RS {R-N-Valery-N-([2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl)valine}, Valsartan related substance II RS {S-N-Butyryl-N-(2'-(1Htetrazol-5-yl)biphenyl-4-yl]methyl)-valine}, Valsartan related substance III RS {S-N-Valery-N-([2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl)-valine benzyl ester}, Vinorelbin Related Substance I {4-O-deacetylvinorelbin}, 5-Vinyl-2-pyrrolidone, Xanthanoic acid, Xanthon, Zolpidem Tartrate related substance I {N,N-Dimethyl-2-[7-methyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-3-yl]acetamide}

2) Reagents and Test Solutions

Acenaphthene $C_{12}H_{10}$ White to pale yellow crystals or crystalline powder with a characteristic aroma. Freely soluble in chloroform or ether, soluble in acetonitrile, sparingly soluble in methanol, and practically insoluble in water.

Identification: Perform the test with 5 mg of Acenaphthene as directed in the paste method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1605 cm^{-1}, 840 cm^{-1}, 785 cm^{-1}, and 750 cm^{-1}.

Melting point: 93 to 96℃

Purity: Dissolve 0.1 g of Acenaphthene in 5 mL of chloroform, and use this solution as the test solution. Perform the test with 2 μL of this solution as directed under the Gas Chromatography according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of acenaphthene by the percentage peak area method; NLT 98.0%.

Operating conditions

Detector: Flame ionization detector

Column: A column about 3 mm in internal diameter and about 2 m in length, packed with 150 to 180 μm diatomaceous earth for gas chromatography, coated with polyethylene glycol 20 M for gas chromatography in a ratio of 10%.

Column temperature: Constant temperature around 210 °C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acenaphthene is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acenaphthene obtained from 2 μL of a solution, prepared by adding chloroform to 1.0 mL of the test solution to make 100 mL, is 5 to 15% of the full scale.

Time span of measurement: About 3 times the retention time of acenaphthene after the solvent peak.

Residue on ignition: NMT 0.1% (1 g).

Acetal $C_6H_{14}O_2$ A colorless and clear, volatile liquid. Miscible with water or ethanol.

Refractive index n: About 1.382

Boiling point: About 103 ℃

Specific gravity d: About 0.824

Acetaldehyde CH_3CHO [First class]

Acetaldehyde ammonium trimer trihydrate $(C_2H_5N)_3 \cdot 3H_2$ Colorless or white to pale yellow crystals or powder.

Content: NLT 95.0%

Assay: Weigh accurately 0.9 g of acetaldehyde ammonium Trimer trihydrate, dissolve in water to make 50 mL, and titrate with 1 mol/L hydrochloric acid (potentiometric titration).

Each mL of 1 mol/L hydrochloric acid
= 61.08 mg of $(C_2H_5N)_3 \cdot 3H_2O$

Acetanilide C_8H_9NO White, crystals or crystalline powder

Melting point: 113 to 116 ℃

***p*-Acetanisidide** $C_9H_{11}NO_2$ White to purplish white crystals or crystalline powder, having a characteristic odor. It is freely soluble in ethanol (95) and in acetonitrile, and very slightly soluble in water.

Melting point: 126 to 132 ℃

Content: NLT 98.0%

Assay: Dissolve about 0.1 g of *p*-acetanisidide in 5 mL of ethanol (95). Perform the test with 3 μL of this solution as directed under the Gas Chromatography according to the following conditions: Determine the area of each peak by the automatic integration method. The peak area of *p*-acetanisidide is NLT 98.0% of the total peak area.

Operating conditions

Detector: Flame ionization detector

Column: A glass column about 3 mm in the internal diameter and about 2 m in the length, packed with 177 to 125 μm acid treated and silylated diatomaceous earth for gas chromatography coated with alkylene glycol phthalate for gas chromatography in the ratio of 1%.

Column temperature: Constant temperature around 210 ℃

Carrier gas: Nitrogen

Flow rate: About 30 to 50 mL per minute (The retention time of *p*-acetanisidide should be about 11 to 14 minutes).

Time span of measurement: About 3 times the retention time of *p*-acetanisidide after the solvent peak.

Acetate buffer solution, 0.05 mol/L, pH 4.0 Dissolve 3.0 mL of acetic acid (100) in 900 mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

Acetate buffer solution, 0.1 mol/L Add 0.1 mol/L sodium acetate solution to 0.1 mol/L hydrochloric acid to adjust the pH to 2.0 (gastropylore powder).

Acetate buffer solution, 0.1 mol/L, pH 4.0 Add 5.1 mL of acetic acid (100) and water to 2.04 g of sodium acetate trihydrate to make 1000 mL.

Acetate buffer solution, 0.1 mol/L, pH 4.7 Dissolve 13.6 g of sodium acetate trihydrate in water, adjust the pH to 4.7 with acetic acid (100), and add water to make 1 L.

Acetate buffer solution, 0.4 mol/L, pH 4.5 Add 54.4 g of sodium acetate and 24 mL of acetic acid (100), and add water to make 1000 mL.

Acetate buffer solution, pH 2.8 Dissolve 4 g of sodium acetate in 800 mL of water, adjust the pH to 2.8, and add water to make 1 L.

Acetate buffer solution, pH 3.5 Dissolve 25 g of ammonium acetate in 25 mL of water, add 38 mL of 7 mol/L hydrochloric acid TS, adjust the pH to 3.5 by adding 2 mol/L hydrochloric acid TS or 6 mol/L ammonia water, and then add water to make 100 mL.

Acetate buffer solution, pH 3.9 Add 10 mL of sodium acetate TS to 60 mL of dilute acetic acid, and add water to make 200 mL.

Acetate buffer solution, pH 4.0 Add 115 mL of acetic acid (100) to 60 g of sodium acetate, and add water to make 1 L.

Acetate buffer solution, pH 4.5 Dissolve 63 g of anhydrous sodium acetate in about 900 mL of water, add 90 mL of acetic acid (100), and then add water to make 1000 mL.

Acetate buffer solution, pH 4.5 Dissolve 120 mL of acetic acid (100) in 83 g of sodium acetate, and add water to make 1 L.

Acetate buffer solution, pH 4.5 Weigh 2.99 g of sodium acetate, dissolve in 500 mL of water, add 1.66 mL of acetic acid, add water to make 1000 mL, and adjust pH to 4.5 with 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide solution (triflusal capsule).

Acetate buffer solution, pH 4.8 Mix 1 mol/L acetic acid and 1 mol/L sodium acetate TS to adjust the pH to 4.8.

Acetate buffer solution, pH 5.0 Add 60 mL of 1 mol/L acetic acid and water to 140 mL of 1 mol/L sodium acetate TS to make 1 L.

Acetate buffer solution, pH 5.2 Mix 79 mL of sodium acetate TS (solution made by dissolving 13.6 g of sodium acetate hydrate in 100 mL of water) and 21 mL of 1 mol/L acetic acid, and add water to make 500 mL. If necessary, adjust the pH to 5.2 ± 0.1 by adding 1 mol/L acetic acid or sodium acetate TS (conjugated estrogen).

Acetate buffer solution, pH 5.5 Dissolve 2.72 g of sodium acetate trihydrate in water to make 1000 mL, and adjust the pH to 5.5 with diluted acetic acid (3 in 2500).

Acetate buffer solution, pH 6.0 Add 40 mL of water to 20 g of sodium acetate, add 3 mL of dilute acetic acid (1 in 100) to adjust the pH to 6.0, and add water again to make 1 L.

Acetate buffer solution, pH 7.5 Weigh 5.9 g of ammonium acetate, dissolve in 760 mL of water, add 1 mL of diethylamine, and adjust the pH to 7.5 with acetic acid (nizatidine).

Acethydrazide trimethylammonium chloride
$[(CH_3)_3N^+CH_2\,CONHNH_2]Cl^-$ Colorless or white crystals, freely soluble in water, practically insoluble in chloroform and hygroscopic One gram of Acethydrazide Trimethylammonium Chloride dissolves in about 25 mL of ethanol.

Melting point: 185 ℃ to 192 ℃ (If necessary, recrystallize in hot ethanol for measurement.)

Acetic acid See acetic acid (31).

Acetic acid (100) CH_3COOH [Special class].

Acetic acid (100) for non-aqueous titration See acetic acid, for non-aqueous titration.

Acetic acid (100) for non-aqueous titration See acetic acid, for non-aqueous titration.

Acetic acid (100)-sulfuric acid TS To 5 mL of acetic acid (100), add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

Acetic acid (31) Dilute 31.0 g of acetic acid (100) with water to make 100 mL (5 mol/L).

Acetic acid buffer solution, pH 4.5 Dissolve 10.25 g of anhydrous sodium acetate in 300 mL of water, and add 10 mL of acetic acid (100) and water to make 1000 mL (Indobufen).

Acetic acid for non-aqueous titration See acetic acid, for non-aqueous titration.

Acetic acid TS, 0.25 mol/L Dilute 3 g of acetic acid (100) with water to make 200 mL.

Acetic acid TS, 6 mol/L Dilute 36 g of acetic acid (100) with water to make 100 mL.

Acetic acid-ammonium acetate buffer solution, pH 3.0 Add acetic acid (31) to ammonium acetate TS to adjust the pH to 3.0.

Acetic acid-ammonium acetate buffer solution, pH 3.0 See acetic acid-ammonium acetate buffer solution, pH 3.0.

Acetic acid-ammonium acetate buffer solution, pH 4.0 Dissolve 7 g of ammonium acetate in 1000 mL of water, and add acetic anhydride to adjust the pH to 4.0.

Acetic acid-ammonium acetate buffer solution, pH 4.5 Dissolve 77 g of ammonium acetate in 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000 mL.

Acetic acid-ammonium acetate buffer solution, pH 4.5 See acetic acid-ammonium acetate buffer solution, pH 4.5.

Acetic acid-ammonium acetate buffer solution, pH 4.8 See acetic acid-ammonium acetate buffer solution, pH 4.8.

Acetic acid-ammonium acetate buffer solution, pH 4.8 Dissolve 77 g of ammonium acetate in about 200 mL of water, and add 57 mL of acetic acid (100) and water to make 1000 mL.

Acetic acid-ammonium acetate buffer solution, pH 6.0 Dissolve 50 g of ammonium acetate in 150 mL of water, adjust the pH to 6.0 with acetic acid (100), and add water to make 250 mL.

Acetic acid-ammonium acetate TS Dissolve 150 g of ammonium acetate in water, add 3 mL of acetic acid (100), then add water to make 1000 mL.

Acetic acid-methanol solution A mixture of 70% methanol and acetic acid (10 : 1)

Acetic acid-potassium acetate buffer solution, pH 4.3 Dissolve 14 g of potassium acetate and 20.5 mL of acetic acid (100) in water to make 1000 mL.

Acetic acid-potassium acetate buffer solution, pH 4.3 See acetic acid-potassium acetate buffer solution, pH 4.3.

Acetic acid-sodium acetate buffer solution for iron test, pH 4.5 To 80 mL of sodium acetate TS, add 120 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, 0.05 mol/L, pH 4.0 To a 1000 mL solution made by adding water to 3.0 g of acetic acid (100), add a 500 mL solution made by dissolving 3.4 g of sodium acetate trihydrate in water to adjust the pH to 4.0.

Acetic acid-sodium acetate buffer solution, 1 mol/L, pH 5.0 To sodium acetate TS, add dilute acetic acid to adjust the pH to 5.0.

Acetic acid-sodium acetate buffer solution, pH 3.8 Dissolve 13.61 g of sodium acetate trihydrate in water and add 60 mL of acetic acid (100) and water to make 1 L.

Acetic acid-sodium acetate buffer solution, pH 4.0 Dissolve 5.44 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.0 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.5 See acetic acid-sodium acetate buffer solution, pH 4.5, for iron test.

Acetic acid-sodium acetate buffer solution, pH 4.5, for iron test Dissolve 75.4 mL of acetic acid (100) and 111 g of sodium acetate trihydrate in water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.7 Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.0 To 140 mL of sodium acetate TS, add 60 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.0 See acetic acid-sodium acetate buffer solution, pH 5.0.

Acetic acid-sodium acetate buffer solution, pH 5.4 Dissolve 20 g of sodium acetate in 80 mL of water, adjust the pH to 5.4 by adding acetic acid (100) dropwise, and add water to make 100 mL.

Acetic acid-sodium acetate buffer solution, pH 5.4 See acetic acid-sodium acetate buffer solution, pH 5.4.

Acetic acid-sodium acetate buffer solution, pH 5.5 Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

Acetic acid-sodium acetate buffer solution, pH 5.6 Dissolve 12 g of sodium acetate with 0.66 mL of acetic acid (100) and water to make 100 mL.

Acetic acid-sodium acetate TS Mix 17 mL of 1 mol/L sodium hydroxide solution with 40 mL of dilute acetic acid, and add water to make 100 mL.

Acetic acid, dilute Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

Acetic acid, for non-aqueous titration Acetic acid (100) that satisfies the following requirements.

Acetic anhydride $(CH_3CO)_2O$ [Special class]

Acetic anhydride-pyridine TS Place 25 g of acetic anhydride in a 100-mL volumetric flask, add pyridine to make 100 mL. Mix well and preserve in light-resistant containers, protected from air. This solution becomes colored during storage but it may still be used.

Acetone CH_3COCH_3 [Special class].

Acetone for non-aqueous titration See acetone, for non-aqueous titration.

Acetone for penicillin See Acetone, for penicillin.

Acetone, for non-aqueous titration Add potassium permanganate to acetone in small portions, shake to mix, and allow the mixture to stand for 2 to 3 days. Once the purple color of the solution disappears, distill it, and dry it with freshly ignited anhydrous potassium carbonate. Distill using a fractionating column under protection from moisture, and collect the fraction distilled at 56 ℃.

Acetone, for penicillin Take an appropriate amount of acetone for non-aqueous titration and add N-ethylpiperidine benzylpenicillin to make a saturated solution. Preserve this solution at 0 to 8 ℃ and use within 3 days after preparation. When performing the assay of the penicillin G sodium RS, the content of penicillin G sodium should be NLT 97%. For use in testing, take it by aspiration with a cotton plug on the tip of a pipet.

Purity *Acetic anhydride*: Dissolve 1.0 g of aniline with acetic acid, for non-aqueous titration to make 100 mL, and use this solution as the test solution. Pipet 25 mL of the test solution, titrate it with 0.1 mol/L perchloric acid VS, and designate the consumed volume as A (mL). A is NLT 26 mL (potentiometric titration). Then, pipet 25 mL of the test solution, add 75 mL of acetic acid, for non-aqueous titration, titrate the resulting solution with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL). A (mL) - B (mL) is NMT 0.1 (mL) (NMT 0.001 g/dL).

Acetonitrile CH_3CN [Special class].

Acetylacetone $CH_3COCH_2COCH_3$ [Special class]

Acetylacetone TS Dissolve 150 g of ammonium acetate in a sufficient quantity of water, add 3 mL of acetic acid (100) and 2 mL of acetylacetone, and add water to make 1000 mL. Prepare before use.

Acetylation TS (acid-alkaline titration) Add 30 mL of anhydrous acetic acid and 0.4 mL of 12% perchloric acid to 180 mL of ethyl acetate, shake for 5 hours, allow to stand for 3 to 4 hours, and then use (dihydroxydibutylether).

Acetylation TS (assay by hydroxyl group) Dissolve 100 g of boron trifluoride in acetic acid (100), add 1 to 2 mL of water, and add acetic acid (100) to make 1 L (dihydroxydibutylether).

Acetylene See dissolved acetylene

Acid-treated gelatin See gelatin, acid-treated.

Acidic ammonium iron(III) sulfate TS See ammonium iron(III) sulfate TS, acidic.

Acidic ammonium sulfate(III) TS See ammonium iron(Ⅲ) sulfate TS, acidic.

Acidic ferrous ammonium sulfate TS See ammonium iron(Ill) sulfate TS, acidic.

Acidic ferrous ammonium sulfate TS (fibracillin) See ammonium iron(III) sulfate TS, acidic (fibracillin).

Acidic ferrous chloride TS See iron(III) chloride TS, acidic.

Acidic iron(III) chloride TS See iron(III) chloride TS, acidic

Acidic methanol To 60 mL of methanol, add carefully 1 mL of sulfuric acid, mix, and add methanol to make 100 mL (loperamide hydrochloride).

Acidic methanol TS To 60 mL of methanol, add carefully 1.85 g of sulfuric acid, and add methanol to make 100 mL.

Acidic potassium chloride solution See potassium chloride TS, acidic.

Acidic potassium chloride TS See potassium chloride TS, acidic.

Acidic potassium permanganate TS See potassium permanganate TS, acidic.

Acidic stannous chloride TS See tin(II) chloride TS, acidic.

Acrinol $C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$ [See monograph, Part II on Acrinol Hydrate]

Activated alumina Aluminum oxide with specially strong adsorptive activity.

Activated charcoal [See monograph, Part I on Medicinal Carbon]

Adipic acid $C_4H_8(COOH)_2$ White crystals or crystalline powder. Freely soluble in ethanol, and slightly soluble in water.

Melting point: 151 to 154 ℃

Content NLT 98.0%.

Assay: Weigh accurately about 1 g of adipic acid, add 100 mL of water, and warm the mixture to dissolve. Allow it to cool down, and titrate the resulting solution with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 73.07 mg of $C_6H_{10}O_4$

Agar [Special class, See monograph, Part II on Agar or Agar Powder. Loss on drying is NMT 15%].

Agar medium, ordinary See ordinary agar medium.
Spot about 10 mL each of ordinary agar medium into agar slant test tubes, and sterilize in an autoclave. Before the medium congeals, allow to stand in a slanting position, and solidify. When the coagulating water is lost, prepare by dissolving with the aid of heat.

Agar slant Dispense portions of about 10 mL of ordinary agar medium into test tubes, and sterilize by autoclaving. Before the medium congeals, allow the test tube to stand in a slanting position, and solidify. When the coagulating water is lost, prepare by dissolving with the aid of heat.

***L*-Alanine** $C_3H_7NO_2$ [Special class]

Albumin TS Carefully separate the whites from 1 fresh egg. Mix with 100 mL of water until two components are thoroughly mixed, and filter. Prepare before use.

Alcoholic hydroxylamine TS See hydroxylamine TS, alcoholic.

Alcoholic phenolphthalein solution, 0.1% Dissolve 10 mg of phenolphthalein in 100 mL of ethanol (95) (DL-phosphoserine).

Alcoholic sulfuric acid solution, 2% Dilute about 50 mL of ethanol with 2 mL of sulfuric acid, and add ethanol to make 100 mL.

Assay: Dissolve 20 mg of Aldehyde Dehydrogenase, accurately weighed, in 1 mL of water, add bovine serum albumin (1 in 100) to make exactly 200 mL, and use this solution as the test solution. Dissolve 2.50 mL of pyrophosphate buffer solution, pH 9.0, and 20 mg of β-nicotinamide adenine dinucleotide (β-NAD) in water to make exactly 1 mL to prepare 0.2 mL pyrazole solution (17 in 2200). Take 0.10 mL of this solution to place in a spectrophotometric cell, add 0.1 mL of the test solution, mix, stopper tightly, and allow to stand at 25 ± 1 ℃ for 2 minutes. To this solution, add 0.01 mL of an acetaldehyde solution (3 in 1000), stir to mix, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under the Ultraviolet-visible Spectroscopy, and calculate a change (ΔA) in absorbance per minute starting from the spot where the relation of time and absorbance is shown with a straight line. One enzyme activity unit means an amount of enzyme which oxidizes 1 μmol of acetaldehyde per minute when the test is performed under the operating conditions of Procedure.

$$\text{Enzyme activity unit of this Reagent (mg)} = \frac{2.91 \times \Delta A \times 200}{6.3 \times W \times 0.10 \times 1000}$$

W: Amount of sample taken (g)

Aldehyde Dehydrogenase Each mg of Aldehyde Dehydrogenase contains NLT 2 enzyme activity units. White powder.

Aldehyde dehydrogenase TS Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10 mL of water. Prepare before use.

Aldehyde-free ethanol See ethanol, aldehyde-free.

Alizarin complexone $C_{19}H_{15}NO_8$ (1,2-dihydroxyanthraqui-

none-3-ylmethylamine-*N*,*N*-diacetate) Yellowish brown powder. Soluble in ammonia TS, and practically insoluble in water, ethanol or ether.

Sensitivity: Dissolve 0.1 g of Alizarin Complexone by adding 2 drops of ammonia water (28), 2 drops of ammonium acetate TS, and 20 mL of water. To 10 mL of this solution, add acetic acid-potassium acetate buffer solution, pH 4.3, to make 100 mL. Place 1 drop of this solution on a white spot plate, add 1 drop of a solution of sodium fluoride (1 in 100,000) and 1 drop of cerium (III) nitrate TS, stir for mixing, and observe under scattered light after 1 minute: a blue-purple color is produced, and the color of the control solution is reddish purple. Prepare a control solution in the same way, except that 1 drop of water is added instead of sodium fluoride solution.

Alizarin complexone TS Dissolve 0.39 g of alizarin complexone in 20 mL of a freshly prepared solution of sodium hydroxide (1 in 50), add 800 mL of water and 0.2 g of sodium acetate trihydrate to dissolve, adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid, and add water to make 1000 mL.

Alizarin red S $C_{14}H_7NaO_7S \cdot H_2O$ [Special class]

Alizarin red S TS Dissolve 0.1 g of alizarin red S in water to make 100 mL. Filter, if necessary.

Alizarin S See alizarin red S.

Alizarin S TS See alizarin red S TS.

Alizarin yellow GG $C_{13}H_{18}N_3NaO_5$ [Special class]

Alizarin yellow GG TS Dissolve 0.1 g of alizarin yellow GG with 100 mL of ethanol. If necessary, filter it.

Alizarin yellow GG-thymolphthalein TS Mix 10 mL of alizarin yellow GG TS with 20 mL of thymolphthalein TS.

Alkaline 1,3-Dinitrobenzene TS See 1,3-Dinitrobenzene TS, alkaline

Alkaline 2,4,6-trinitrophenol TS See 2,4,6-trinitrophenol TS, alkaline.

Alkaline blue tetrazolium TS See blue tetrazolium TS, alkaline.

Alkaline copper solution, alkaline copper solution Dissolve 0.4 g of sodium hydroxide in 80 mL of water, add 2 g of anhydrous sodium carbonate and water to make 100 mL, and use this solution as Solution A. Separately, dissolve 1 g of sodium tartrate in 80 mL of water, add 0.5 g of copper (II) sulfate pentahydrate and water to make 100 mL, and use this solution as Solution B. Add 1 mL of Solution B to 50 mL of Solution A for use (sodium hyaluronate, sodium hyaluronate for ocular injection, elcatonin).

Alkaline copper sulfate TS See copper(II) sulfate TS, alkaline.

Alkaline copper TS Dissolve 70.6 g of disodium hydrogen phosphate 12-hydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600 mL of water, and add 20 mL of sodium hydroxide solution (1 in 5). To this mixture, while stirring, add 100 mL of copper sulfate solution (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate and water to make 1000 mL.

Alkaline copper TS See copper TS, alkaline.

Alkaline glycerin TS To 200 g of glycerin, add water to make 235 g, and then dissolve in 142.5 mL of sodium hydroxide TS and 47.5 mL of water.

Alkaline hydroxylamine TS See hydroxylamine TS, alkaline.

Alkaline *m*-dinitrobenzene TS See 1,3-dinitrobenzene TS, alkaline.

Alkaline phosphatase See Phosphatase, alkaline

Alkaline phosphatase enzyme [First class]

Alkaline phosphatase TS Add magnesium buffer solution, pH 9, to 95 ± 5 mg of alkaline phosphatase enzyme to make 50 mL. Prepare before use.

Alkaline picric acid TS See picric acid TS, alkaline.

Alkaline potassium hexacyanoferrate(III) TS See potassium hexacyanoferrate(III) TS, alkaline.

Alkaline sodium pentacyanonitrosylferrate(III) solution Dissolve 1 g of sodium pentacyanonitrosylferrate(III) dihydrate and 1 g of anhydrous sodium carbonate in water to make 100 mL.

Alkylene glycol phthalate ester for gas chromatography Prepared for gas chromatography.

Aluminium potassium sulfate $K_2Al_2(SO_4)_4 \cdot 24H_2O$ [aluminium potassium sulfate (potassium alum), Special class]

Aluminium potassium sulfate dodecahydrate $AlK(SO_4)_2 \cdot 12H_2O$ [Special class]

Aluminon $C_{22}H_{23}N_3O_9$ [Special class]

Aluminon TS Dissolve 0.1 g of aluminon in water to make 100 mL. Use it after allowing to stand for 24 hours.

Aluminum Al [Special class] Presented in the forms of thin strips, sheets, wires or thin-layer plates.

Aluminum chloride See aluminum (III) chloride hexahydrate.

Aluminum chloride TS Dissolve 64.7 g of aluminum (III) chloride hexahydrate in 71 mL of water, add 0.5 g of activated charcoal, shake for 10 minutes for mixing, and filter. While stirring the filtrate for mixing, add sodium hydroxide solution (1 in 100) to adjust the pH to 1.5, and if necessary, filter.

Aluminum oxide Al_2O_3 White crystals, crystalline powder or powder.

Melting point: About 2000 ℃
Boiling point: About 3000 ℃

Aluminum(III) chloride hexahydrate $AlCl_3 \cdot 6H_20$ [Special Class]

Amidosulfuric acid (standard reagent) $HOSO_2NH_2$ [Reference material for volumetric analysis]

***N*-Amino hexamethyleneimine** $(CH_2)_6NNH_2$ A clear colorless to pale yellow liquid.

Refractive index n_D^{20}: between 1.482 and 1.487

Specific gravity d_{20}^{20}: 0.936 to 0.942

2-Amino-1-butanol $CH_3CH_2CH(NH_2)CH_2OH$ Clear, colorless to pale yellow liquid. Miscible with water or methanol.

Refractive index n_D^{20}: 1.450 to 1.455

Specific gravity d_{20}^{20}: 0.944 to 0.950

Purity *Related substances*: Weigh 50 mg of 2-amino-1-butanol and dissolve in 10 mL of methanol. Perform the test with 2 μL of this solution as directed in the Purity (4) under Ethambutol Hydrochloride; any spots other than the principal spot with the R_f value of about 0.3 do not appear.

2-Amino-2-hydroxymethyl-1,3-propanediol TS Dissolve 1.5 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 100 mL. Pipet 40 mL of this solution and add dimethylsulfoxide to make 200 mL. Use this TS within 4 hours after preparation.

2-Amino-2-hydroxymethyl-1,3-propanediol $C_4H_{11}NO_3$ [Special class]

1-Amino-2-naphthol-4-sulfonic acid TS Mix thoroughly 5 g of anhydrous sodium sulfite, 94.3 g of anhydrous sodium bisulfite, and 0.7 g of 1-amino-2-naphthol-4-sulfonic acid. Dissolve 1.5 g of this mixture in water before use to make 10 mL.

1-Amino-2-naphthol-4-sulfonic acid $C_{10}H_9NO_4S$ [Special class]

2-Amino-5-chlorobenzophenone for thin layer chromatography See 2-amino-5-chlorobenzophenone, for thin layer chromatography.

2-Amino-5-chlorobenzophenone, for thin layer chromatography $C_{13}H_{10}ClNO$ Yellow crystalline powder.

Melting point: 97 to 101 ℃

Purity *Related substances:* Dissolve 10 mg of 2-amino-5-chlorobenzophenone, for thin layer chromatography in methanol to make exactly 200 mL, and perform the test with this solution as directed in the Purity (3) under Chlordiazepoxide; any spots other than the principal spot with the R_f value of about 0.7 do not appear.

4-Amino-6-chloro-1,3-benzenedisulfonamide $C_6H_8ClN_3O_4S_2$ White, odorless powder. It is practically insoluble in water or chloroform and soluble in ammonium TS. Perform the test with this solution in methanol (1 in 200,000) as directed under the Ultraviolet-visible Spectroscopy; it exhibits a maximum absorption at 223 nm and 265 nm.

Absorbance $E_{1cm}^{1\%}$ (265 nm): About 64

Residue on ignition: NMT 0.1% (2 g).

4-Amino-*N,N*-diethylaniline sulfate monohydrate $H_2NC_6H_4N(C_2H_5)_2 \cdot H_2SO_4 \cdot H_2O$ White to slightly colored powder. Soluble in water.

Melting point: 173 to 176 ℃

Residue on ignition NMT 0.10% (1 g).

4-Amino-*N,N*-diethylaniline sulfate TS Dissolve 0.2 g of 4-amino-*N,N*-diethylaniline sulfate monohydrate in water to make 100 mL. Prepare before use, protected from light.

Aminoacetic acid See glycine.

4-Aminoacetophenone TS Dissolve 0.1 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

***p*-Aminoacetophenone TS** See 4-aminoacetophenone TS.

4-Aminoacetophenone $H_2NC_6H_4COCH_3$ [*p*-aminoacetophenone] Pale yellow crystals or crystalline powder with a characteristic odor.

Melting point: 105 to 108 ℃

***p*-Aminoacetophenone** See 4-aminoacetophenone.

4-Aminoantipyrine hydrochloride $C_{11}H_{13}N_3O \cdot HCl$ A pale yellow crystalline powder. Soluble in water.

Melting point: 232 to 238 ℃ (with decomposition)

Purity *Clarity and color of solution*: Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water; the solution is almost clear.

Content: 100.6 to 108.5%

Assay: Weigh accurately about 0.5 g of 4-aminoantipyrine hydrochloride, dissolve in 50 mL of water. If necessary, neutralize it with 0.1 mol/ L sodium hydroxide solution (indicator: red litmus paper). Then, add 4 drops of dichlorofluorescein TS, and titrate the resulting solution with 0.1 mol/L silver nitrate VS.

Each mL of 0.1 mol/L silver nitrate VS
= 23.970 mg of $C_{11}H_{13}H_3O \cdot HCl$

4-Aminoantipyrine hydrochloride TS Dissolve 1 g of 4-Aminoantipyrine hydrochloride in water to make 50 mL.

Aminoantipyrine TS See 4-aminoantipyrine TS.

4-Aminoantipyrine $C_{11}H_{13}N_3O$ [4-aminoantipyrine(4-amino-1,5-dimethyl-2-phenyl-3H-pyrazolone-3-one), Special class]

4-Aminoantipyrine TS Dissolve 0.1 g of 4-aminoantipyrine in 30 mL of water, add 10 mL of sodium carbonate solution (1 in 5) and 2 mL of sodium hydroxide TS, and then add water to make 100 mL. Prepare before use.

4-Aminoantipyrine TS Add 0.1% hydrochloric acid-methanol solution to 200 mg of 4-aminoantipyrine to make 50 mL (prepare this solution before use) (flumethasone pivalate).

Aminobenzoic acid derivatization TS To 0.28 g of ethyl aminobenzoate, add 600 μL of methanol, dissolve it on a water bath at about 50℃, and add 170 μL of acetic acid (31) and 145 μL of borane-pyridine complex.

4-Aminobenzoic acid A white to pale yellow crystalline powder.

Purity *Clarity and color of solution*: Dissolve 1.0 g of 4-aminobenzoic acid in 10 mL of ethanol; the resulting solution is clear.

***p*-Aminobenzoic acid** See 4-aminobenzoic acid.

2-Aminoethanol $H_2NCH_2CH_2OH$ [Special class].

4-(Aminomethyl)benzoic acid $C_8H_9NO_2$ White powder.

4-Aminophenazone $C_{13}H_{17}N_3O$ [Special class]

4-Aminophenazone TS Dissolve 0.30 g of 4-aminophenazone in 50 mL of methanol acidified with 1.75 mL of hydrochloric acid. Prepare before use (clobetasol propionate).

4-Aminophenol hydrochloride $HOC_6H_4NH_2 \cdot HCl$ White to pale colored crystals. Freely soluble in water and ethanol (95).

Melting point: About 306℃

Content: NLT 99.0%.

Assay: Weigh accurately about 0.17 g of 4-aminophenol hydrochloride, add 50 mL of acetic acid for non-aqueous titration and 5 mL of mercury(II) acetate TS for non-aqueous titration to dissolve, and titrate the resulting solution with 0.1 mol/L perchloric acid-dioxane VS (indicator: 1 mL of *p*-naphtholbenzein TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 14.559 mg of $HOC_6H_4NH_2 \cdot HCl$

Packaging and storage: Preserve in light-resistant, tight containers.

***p*-Aminophenol hydrochloride** See 4-aminophenol hydrochloride.

***p*-Aminophenol hydrochloride** See 4-aminophenol hydrochloride.

3-Aminophenol $H_2NC_6H_4OH$ White crystals or crystalline powder.

Melting point: 121 to 125 ℃

Content: NLT 97.0%.

Assay: Weigh accurately about 0.2 g of 3-aminophenol, dissolve in 50 mL of acetic acid for non-aqueous titration, and titrate the resulting solution with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.91 mg of $H_2NC_6H_4OH$

***m*-Aminophenol** See 3-aminophenol.

***p*-Aminophenol** C_6H_7NO Fine, yellow crystalline powder. Slightly soluble in water and ethanol.

Melting point: 187 to 189 ℃

Aminopropylsilyl silica gel for liquid chromatography Prepared for liquid chromatography.

Aminopropylsilyl silica gel, for liquid chromatography Prepared for liquid chromatography.

***L*-2-aminosuberic acid** $C_8H_{15}NO_4$ White crystals or crystalline powder, which is odorless.

Specific optical rotation $[\alpha]_D^{20}$: +19.1 to +20.1° (After drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm)

Loss on drying: NMT 0.3% (1 g, 105 ℃, 2 hours)

Assay: Weigh accurately about 0.3 g of L-2-aminosuberic acid, previously dried, and add exactly 6 mL of formic acid to dissolve. Then, add exactly 50 mL of acetic acid (100), and titrate the resulting solution with 0.1 mol/L perchloric acid VS (potentiometric titration under the Titrimetry). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.92 mg of $C_8H_{15}NO_4$

Ammonia copper TS Grind 0.5 g of copper carbonate monohydrate with 10 mL of water, and add 10 mL of ammonia water (28).

Ammonia gas NH_3 Prepare by heating ammonia water (28).

Ammonia TS Add water to 400 mL of ammonia water (28) to make 1000 mL (10%).

Ammonia water See ammonia TS.

Ammonia water (28) NH_4OH [Ammonia water, Special class, density 0.908 g/mL, content 28 to 30%]

Ammonia water, 0.5 mol/L Add water to 32.5 mL of ammonia water (28) to make 1000 mL.

Ammonia water, 1 mol/L Add water to 65 mL of ammonia water (28) to make 1000 mL.

Ammonia water, 13.5 mol/L Take exactly 9 mL of water, and add ammonia water (28) to make exactly 50 mL.

Ammonia water, strong See ammonia water (28).

Ammonia-ammonium acetate buffer solution, pH 8.0 Add ammonia TS dropwise to ammonium acetate TS to adjust the pH to 8.0.

Ammonia-ammonium acetate buffer solution, pH 8.5 Dissolve 50 g of ammonium acetate with 800 mL of water and 200 mL of ethanol (95), and add ammonia water (28) to adjust the pH to 8.5.

Ammonia-ammonium chloride buffer solution, pH 10.0 Dissolve 70 g of ammonium chloride in water, add 100 mL of ammonia water (28), dilute with water to make 1000 mL, and add ammonia water (28) dropwise to adjust the pH to 10.0.

Ammonia-ammonium chloride buffer solution, pH 10.7 Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonia water (28), and dilute with water to make 1000 mL.

Ammonia-ammonium chloride buffer solution, pH 11.0 Dissolve 53.5 g of ammonium chloride in water, add 480 mL of ammonia water (28), and dilute with water to make 1000 mL.

Ammonia-ammonium chloride buffer solution, pH 11.0 Dissolve 53.5 g of ammonium chloride in water, add 480 mL of ammonia water (28), and dilute with water to make 1000 mL.

Ammonia-ammonium chloride buffer solution, pH 8.0 Dissolve 1.07 g of ammonium chloride in water to make 100 mL, and adjust the pH to 8.0 by adding diluted ammonia TS (1 in 30).

Ammonia-cyanide TS Dissolve 2 g of potassium cyanide in 15 mL of ammonia water (28), and dilute with water to 100 mL.

Ammonia-ethanol TS To 20 mL of ammonia water (28), add

100 mL of ethanol (99.5).

Ammonia-methanol solution Dissolve 5 mL of strong ammonia water in methanol to make 100 mL (omeprazole tablet).

Ammonia-methanol TS Add methanol to 20 mL of 25% ammonia to make 1000 mL.

Ammonia-methanol TS, 0.05 mol/L To 0.8 mL of 0.05 mol/L ammonia water, add methanol to make 250.0 mL.

Ammonium acetate CH_3COONH_4 [Special class]

Ammonium acetate buffer solution, 0.02 mol/L, pH 4.0 Weigh 1.54 g of ammonium acetate, dissolve in 900 mL of water, adjust the pH to 4.0 with acetic acid, and then add water to make 1000 mL.

Ammonium acetate buffer solution, 0.1 mol/L, pH 5.7 Dissolve 7.708 g of ammonium acetate in 900 mL of water, adjust the pH to 5.7 with 0.1 mol/L acetic acid, and add water to make 1000 mL.

Ammonium acetate buffer solution, pH 3.5 Dissolve 5.0 g of ammonium acetate in 5.5 mL of hydrochloric acid, and add water to make 20 mL.

Ammonium acetate solution Dissolve 150 g of ammonium acetate in water, add 3 mL of acetic acid (100), and add water to make 1000 mL.

Ammonium acetate solution See ammonium acetate solution.

Ammonium acetate TS Dissolve 10 g of ammonium acetate in water to make 100 mL.

Ammonium acetate TS, 0.5 mol/L Dissolve 38.5 g of ammonium acetate in water to make 1000 mL.

Ammonium acetate-hydrochloric acid buffer solution, pH 3.5 Add about 650 mL of 4 mol/L hydrochloric acid to 250 g of ammonium acetate, and add water to make 1 L.

Ammonium amidosulfate $NH_4OSO_2NH_2$ [Special class]

Ammonium amidosulfate TS Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

Ammonium bicarbonate NH_4HCO_3 [Ammonium bicarbonate, Special Class]

Ammonium carbonate [Special Class]

Ammonium carbonate TS To 20 g of ammonium carbonate, add 20 mL of ammonia TS and water to dissolve and make 100 mL.

Ammonium cerium sulfate See tetraammonium cerium(IV) sulfate dihydrate

Ammonium cerium sulfate TS See tetraammonium cerium (IV) sulfate TS

Ammonium cerium sulfate-phosphoric acid TS See tetraammonium cerium (IV) sulfate-phosphoric acid TS

Ammonium chloride NH_4Cl [Special Class]

Ammonium chloride buffer solution, pH 10 Dissolve 5.4 g of ammonium chloride in water, and add 21 mL of ammonia water (28) and water to make 100 mL.

Ammonium chloride TS Dissolve 10.5 g of ammonium chloride in water to make 100 mL (2 mol/L).

Ammonium chloride-ammonia TS To ammonia water (28), add an equal volume of water, and saturate this solution with ammonium chloride.

Ammonium citrate See diammonium hydrogen citrate.

Ammonium citrate solution Dissolve 40 g of citric acid monohydrate in 90 mL of water, add 2 to 3 drops of phenol red TS, and carefully add ammonia water (28) until a red color appears. Remove lead by extracting with 20 mL of extracting dithizone solution until the extract turns orangish green.

Ammonium dihydrogen phosphate $NH_4H_2PO_4$ [Special Class]

Ammonium dihydrogen phosphate buffer solution, 0.02 mol/L, pH 3.6 Dissolve 2.3 g of ammonium dihydrogen phosphate, accurately weighed, in 950 mL of water, adjust the pH to 3.6 with phosphoric acid, and add water to make 1 L (cloperastine hydrochloride).

Ammonium dihydrogen phosphate TS, 0.01 mol/L Dissolve 1.15 g of ammonium dihydrogen phosphate in water to make 1 L.

Ammonium dihydrogen phosphate TS, 0.02 mol/L Dissolve 2.30 g of ammonium dihydrogen phosphate 2.30 g in water to make 1000 mL.

Ammonium dihydrogen phosphate TS, 0.05 mol/L Dissolve 5.75 g of ammonium dihydrogen phosphate 2.30 g in water to make 1000 mL.

Ammonium ferric citrate See ammonium iron(III) citrate.

Ammonium ferric sulfate See ammonium iron(III) sulfate dodecahydrate.

Ammonium ferric sulfate TS See ammonium iron(III) sulfate TS

Ammonium ferric sulfate TS, dilute See ammonium iron(III) sulfate TS, dilute

Ammonium ferrous sulfate See ammonium iron(II) sulfate hexahydrate

Ammonium formate $HCOONH_4$, [Special class]

Ammonium formate buffer solution Add 20 mL of water to 34 mL of ammonia water, slowly add 30 mL of formic acid while stirring, cool, and add water to make 100 mL (oxomemazine hydrochloride, guaifenesin, acetaminophen, sodium benzoate capsules).

Ammonium formate buffer solution, 0.05 mol/L, pH 4.0 Dissolve 3.25 g of ammonium formate in water to make 1 L and adjust the pH to 4.0 with formic acid.

Ammonium formate TS, 0.05 mol/L Dissolve 3.25 g of ammonium formate in water to make 1000 mL.

Ammonium heptamolybdate(VI) tetrahydrate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ [Special Class]

Ammonium hydrogen sulfate NH_4HSO_4 White crystals. Freely soluble in water; practically insoluble in ethanol, acetone or pyridine.

Content: NLT 98%

Assay: Weigh accurately about 0.3 mg of Ammonium Hydrogen Sulfate, dissolve in 50 mL of a mixture of water and ethanol (25 : 25). Titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.51 mg of NH_4HSO_4

Ammonium hydroxide monohydrate $(NH_4)_2C_2O_4\cdot H_2O$ [Special class].

Ammonium hydroxide TS See ammonium oxalate TS,

Ammonium hydroxide TS, 4% See ammonium oxalate TS, 4%.

Ammonium iron(II) sulfate hexahydrate $FeSO_4(NH_4)_2SO_4\cdot 6H_2O$ [Special class]

Ammonium iron(III) citrate [See monograph in the Food Additives Compendium]

Ammonium iron(III) sulfate dodecahydrate $FeNH_4(SO_4)_2\cdot 12H_2O$ [Special class]

Ammonium iron(III) sulfate TS Dissolve 8 g of ammonium iron(III) sulfate dodecahydrate water to make 100 mL.

Ammonium iron(III) sulfate TS, acidic Dissolve 20 g of ammonium iron(III) sulfate in water, add 9.4 mL of sulfuric acid, and add water to make 100 mL.

Ammonium iron(III) sulfate TS, acidic Add 272 g of ammonium iron(III) sulfate and 26 mL of sulfuric acid to water to make 1000 mL. Store this test solution in a brown bottle at ordinary temperature and use it within one week (fibracillin).

Ammonium iron(III) sulfate TS, dilute To 2 mL of ammonium iron(III) sulfate TS, add 1 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

Ammonium metavanadate TS, 0.25% Weigh 2.5 g of ammonium metavanadate, dissolve in water by warming to make 500 mL, and cool. To this solution, add 10 mL of 60% perchloric acid, and add water to make the entire amount 1 L.

Ammonium molybdate See ammonium heptamolybdate(VI) tetrahydrate.

Ammonium molybdate TS Dissolve 21.2 g of ammonium heptamolybdate(VI) tetrahydrate in water to make 200 mL (10%). Prepare before use.

Ammonium molybdate TS Dissolve 25 g of ammonium heptamolybdate tetrahydrate in 200 mL of water, add 300 mL of 5 mol/L sulfuric acid (fructose diphosphate magnesium hydrate).

Ammonium molybdate TS Dissolve 25 g of ammonium heptamolybdate tetrahydrate in 300 mL of water, add 75 mL of sulfuric acid and 125 mL of water, and shake to mix. If this solution exhibits a yellow color or a yellow precipitate is produced, filter it. Prepare before use (lecithin).

Ammonium molybdate-sulfuric acid TS Dissolve 1.0g of ammonium heptamolybdate(VI) tetrahydrate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

Ammonium nickel(II) sulfate See ammonium nickel(II) sulfate hexahydrate.

Ammonium nickel(II) sulfate hexahydrate $NiSO_4(NH_4)_2SO_4\cdot 6H_2O$ [Special class]

Ammonium nitrate NH_4NO_3 [Special Class]

Ammonium oxalate See ammonium oxalate monohydrate.

Ammonium oxalate monohydrate $(NH_4)_2C_2O_4\cdot H_2O$ [Special Class]

Ammonium oxalate TS Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/L).

Ammonium oxalate TS, 0.1mol/L Dissolve 1.42 g of ammonium oxalate monohydrate in water to make 100 mL.

Ammonium oxalate TS, 4% Dissolve 4 g of ammonium oxalate monohydrate in water to make 100 mL.

Ammonium peroxydisulfate $(NH_4)_2S_2O_8$ [Special class]

Ammonium persulfate See ammonium peroxydisulfate.

Ammonium phosphate buffer solution, pH 6.0, 0.01 mol/L Dissolve 1.15 g of ammonium dihydrogen phosphate in 1000 mL of water and adjust the pH to 6.0 with sodium hydroxide TS (trazodone hydrochloride capsule).

Ammonium polysulfide TS $(NH_4)_2S_n$ [Ammonium sulfate solution (yellow), First class]

Ammonium pyrrolidinedithiocarbamate $C_5H_{12}N_2S_2$ [First class]

Ammonium pyrrolidinedithiocarbamate solution, saturated Prepare by saturating ammonium pyrrolidinedithiocarbamate (containing about 10 g per L).

Ammonium reineckate TS See reinecke salt TS.

Ammonium sulfate $(NH_4)_2SO_4$ [Special class]

Ammonium sulfide TS $(NH_4)_2S$ [First class] Store in small, well-filled, light-resistant bottles.

Ammonium sulfate $(NH_4)_2SO_4$ [Special class]

Ammonium sulfate buffer solution Dissolve 264 g of ammonium sulfate in 1000 mL of water, add 1000 mL of 0.5 mol/L sulfuric acid TS, shake, and filter. The pH of this solution is about 1.

Ammonium sulfide TS $(NH_4)_2S$ [First class] Store in small, well-filled, light-resistant bottles.

Ammonium tartrate See L-ammonium tartrate.

***L*-ammonium tartrate** $C_4H_{12}N_2O_6$ [Special Class]

Ammonium thiocyanate NH_4SCN [Special Class]

Ammonium thiocyanate solution, 3 mol/L Add water to 23 g of ammonium thiocyanate to make 100 mL (zinc pyrithion).

Ammonium thiocyanate TS See ammonium thiocyanate TS.

Ammonium thiocyanate TS Dissolve 8 g of ammonium thiocyanate in water to make 100 mL (1 mol/L).

Ammonium thiocyanate TS, 0.1mol/L Dissolve 8 g of sodium chloride in water to make 1000 mL.

Ammonium thiocyanate-cobalt nitrate TS Dissolve 17.4 g of ammonium thiocyanate and 2.8 g of cobalt (II) nitrate hexahydrate to make 100 mL.

Ammonium vanadate NH_4VO_3 [Special class]

Amphotericin B TS Dissolve 22.5 mg of amphotericin B in 9 mL of sterilize purified water.

Amyl alcohol See *n*-Amyl alcohol.

Amyl alcohol, iso See 3-methyl-1-butanol.

Amyl alcohol, tert See *t*-amyl alcohol.

***n*-Amyl alcohol** $CH_3(CH_2)_4OH$ Clear, colorless liquid with a slight characteristic odor. Sparingly soluble in water and miscible with ethanol (95) and ether.

Refractive index n_D^{20}: 1.409 to 1.411
Specific gravity d_4^{20}: 0.810 to 0.820
Distilling range: 135 to 140 ℃, NLT 95 vol%.

***t*-Amyl alcohol** $(CH_3)_2C(OH)CH_2CH_3$ Clear, colorless liquid, having a characteristic odor. Miscible with *t*-butanol or 2-butanol, and freely soluble in water.

Specific gravity d_{20}^{20}: between 0.808 and 0.815

Purity *Acid and ester*: To 20 mL of this drug, add 20 mL of ethanol (95) and 5.0 mL of 0.1 mol/ L sodium hydroxide solution, and heat gently under a reflux condenser on a water bath for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L hydrochloric acid. Perform a blank determination: NMT 1.25 mL of 0.1 mol/L sodium hydroxide solution is consumed.

Nonvolatile residue: Evaporate 50 mL of this drug, and dry at 105℃ for 1 hour: the residue is NMT 1.6 mg.

Distilling range: 100 to 103 ℃, NLT 95 vol%.

Tert-Amyl alcohol See *t*-amyl alcohol.

Amylose tris(3,5-dimethylphenylcarbamate)-coated silica gel for liquid chromatography Prepared for liquid chromatography.

Anesthetic ether See ether, for anesthetia.

Anhydrous benzene See benzene, anhydrous.

Anhydrous caffeine See caffeine, anhydrous.

Anhydrous copper(II) sulfate See copper(II) sulfate, anhydrous.

Anhydrous diethyl ether See ether, anhydrous.

Anhydrous disodium hydrogen phosphate See disodium hydrogen phosphate, anhydrous.

Anhydrous ethanol See ethanol (99.5).

Anhydrous ether See ether, anhydrous.

Anhydrous ferrous chloride-pyridine TS See iron(III) chloride-pyridine TS, anhydrous.

Anhydrous formic acid See formic acid, anhydrous

Anhydrous iron(III) chloride-pyridine TS See iron(III) chloride-pyridine TS, anhydrous.

Anhydrous lactose $C_{12}H_{22}O_{11}$ [See monograph, Part II on Anhydrous Lactose].

Anhydrous methanol See methanol, anhydrous.

Anhydrous phthalic acid See phthalic acid, anhydrous.

Anhydrous potassium carbonate See potassium carbonate, anhydrous.

Anhydrous pyridine See pyridine, anhydrous.

Anhydrous sodium acetate See sodium acetate, anhydrous.

Anhydrous sodium bisulfite See sodium bisulfite, anhydrous.

Anhydrous sodium carbonate See sodium carbonate, anhydrous.

Anhydrous sodium monohydrogen phosphate See disodium hydrogen phosphate, anhydrous.

Anhydrous sodium monohydrogen phosphate for pH measurement See disodium hydrogen phosphate, for pH measurement.

Anhydrous sodium monohydrogen phosphate, for pH measurement See sodium monohydrogen phosphate, for pH measurement.

Anhydrous sodium sulfate See sodium sulfate, anhydrous.

Anhydrous sodium sulfite See sodium sulfite, anhydrous.

Anhydrous succinic acid See succinic acid, anhydrous.

Anhydrous trifluoroacetic acid for gas chromatography **$(CF_3CO)_2O$** Colorless and transparent liquid, having a pungent odor.

Boiling point: 40 ℃ to 45 ℃

Aniline $C_6H_5NH_2$ [Special class]

Aniline TS Add 50 mL of dilute acetic acid (10 in 100) to 0.3 mL of aniline, and mix it with the same volume of potassium persulfate solution (2 in 100).

***p*-Anisaldehyde-acetic acid TS** See 4-methoxybenzaldehyde-acetic acid TS.

***p*-Anisaldehyde** See 4-methoxybenzaldehyde.

***p*- Anisaldehyde-sulfuric acid TS** See 4-methoxybenzaldehyde-sulfuric acid TS.

***p*-Anisidine** C_7H_9NO [First class]

Anisole C_7H_8O A colorless liquid. Boiling point is about 155℃.

Specific gravity d_{20}^{20}: 0.995 to 1.001

Anthrone $C_{14}H_{10}O$ [Special class]

Anthrone solution Put 0.2 g of anthrone in a 100-mL volumetric flask, wash down the anthrone on the flask wall with 20 mL of water, and slowly add about 60 mL of sulfuric acid while cooling. Shake to mix until anthrone is dissolved, cool, and then add sulfuric acid to make 100 mL.

Anthrone TS Dissolve 35 mg of anthrone in 100 mL of sulfuric acid. Prepare before use.

Anti-A blood typing antibody Suitable for the standards of antibodies for blood determination.

Anti-B blood typing antibody Suitable for the standards of antibodies for blood determination.

Anti-bradykinin Immunize a rabbit with bradykinin to obtain antiserum and use it to make antibodies. Dissolve this antibody in 0.04 mol/L phosphate buffer solution (pH 7.0) containing 1 mg/mL bovine serum albumin. The resulting liquid is colorless to pale brown. Growth promotion: Take an appropriate amount of Anti-bradykinin, and add 0.04 mol/L phosphate buffer solution (pH 7.0) containing 1 mg/mL bovine serum albumin to make a 1 vol% solution. With 0.1 mL of this solution, determine the absorbance A_1 and A_2 of standard solution (1) and standard solution (7) at the wavelengths of 490 to 492 nm, as directed under Purity (2) for Kallidinogenase. A_2 - A_1 are NLT 1.

Anti-bradykinin antibody TS Dissolve 0.15 mL of anti-bradykinin antibody, 15 mg of bovine serum albumin, 2.97 mg of sodium dihydrogen phosphate dihydrate, 13.5 mg of disodium hydrogen phosphate dodecahydrate, and 13.5 mg of sodium chloride in water to make 15 mL. After freeze-drying, add 15 mL of water to dissolve. Prepare before use.

Anti-urokinase serum Using urokinase, which contains more than 140000 units per mg of protein, add isotonic sodium chloride injection so that it contain 1 mg of protein per each mL, add the same amount of Freund Complete Adjuvant, and emulsify. Inject 2 mL of this solution intradermally into a healthy rabbit weighing 2.5 to 3.0 kg three times at weekly intervals. 7 to 10 days after the last injection, draw blood to obtain antiserum.

Antimony(V) chloride $SbCl_5$ [Special Class]

Antimony pentachloride See antimony (V) chloride.

Antimony pentachloride TS A solution made by dissolving 2 mg of antimony pentachloride in 8 mL of chloroform (ergocalciferol, retinol acetate).

Antimony potassium tartrate $C_4H_4KO_7Sb \cdot 1/2H_2O$ [Special Class]

Antimony trichloride See antimony(III) chloride.

Antimony trichloride TS See Antimony(III) chloride TS.

Antimony(III) chloride $SbCl_3$ [Special Class]

Antimony(III) chloride TS Wash chloroform with an equal volume of water two or three times, add freshly ignited and cooled potassium carbonate, stopper, and allow to stand overnight, protected from light. Separate the chloroform layer, and distill it, preferably while protected from light. With this chloroform, wash the surface of antimony (III) chloride until the washings become clear and transparent, add the chloroform to make a saturated solution, and place in a light-resistant, stoppered bottle. Prepare before use.

Antimony(III) chloride TS, 15% Dissolve 15 g of antimony (III) chloride in perchloric acid to make 100 mL (ginseng extracts G115).

***β*-Apo-oxytetracycline** $C_{22}H_{22}N_2O_8$ Yellowish brown to brown powder.

Purity *Related substances*: Dissolve 8 mg of β-Apo-oxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide solution, then add 0.01 mol/L hydrochloric acid solution to make 100 mL, and use this solution as the test solution. Measure each peak area by the automatic integration method and percentage peak area method with 20 μL of this solution as directed in the Purity (1) under Oxytetracycline hydrochloride; the sum of peak areas other than *β*-apo-oxytetracycline is NMT 10%.

Aprotinin A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cattle. The pH is 5.0 to 7.0.

Content: Contains 15000 to 25000 KIE units of aprotinin per mL.

Aprotinin TS Weigh an appropriate amount of aprotinin, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to prepare a solution containing 50 KIE Units per mL.

Aqua regia Mix 3 volumes of hydrochloric acid and 1 volume of nitric acid. Prepare before use.

***L*-Arginine hydrochloride** $C_6H_{14}N_4O_2 \cdot HCl$ [See monograph, Part I]

***L*-Arginine** $C_6H_{14}N_4O_2$ White crystals or crystalline powder with a characteristic odor.

Specific optical rotation $[\alpha]_D^{20}$: +26.9 to +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50 mL, 200 mm)

Loss on drying: NMT 0.50% (1 g, 105℃, 3 hours)

Content: 98.0 to 102.0%

Assay: Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate the resulting solution with 0.1 mol/L perchloric acid VS (indicator: 10 drops of *p*-naphtholbenzein TS). The endpoint of the titration is when the color of the solution changes from yellowish brown to yellow, and then to green. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.710 mg of $C_6H_{14}N_4O_2$

Arsenazo III $C_{22}H_{18}As_2N_4O_{14}S_2$ [Special class]

Arsenazo III TS Weigh 0.1 g of aresenazo III and add water to make 50 mL.

Arsenic free zinc oxide See zinc, for arsenic analysis.

Arsenic hydrogen absorbing solution Dissolve 0.50 g of silver diethyldithiocarbamate in pyridine to make 100 mL. Preserve in stoppered bottles in a cold place, protected from light.

Arsenic molybdate TS Weigh accurately 50.0 g of ammonium molybdate, add 900 mL of water, and dissolve by warming. After cooling, add 42.0 mL of sulfuric acid and 50 mL of sodium monohydrogen arsenic acid, add water to make 1 L, allow to stand at 37 ℃ for 24 hours, and preserve in brown bottles.

Arsenic trioxide AS_2O_3 [Arsenic trioxide(arsenic), Special class].

Arsenic trioxide (standard reagent) [Reagent for volumetric analysis].

Arsenic trioxide TS To 1 g of arsenic trioxide, add 30 mL of a solution of sodium hydroxide (1 in 40), dissolve by heating, cool, and add gently acetic acid (100) to make 100 mL.

Arsenous acid TS, 0.01 mol/L Dissolve 1.9 g of arsenic trioxide (As_2O_3) in 10 mL of 2 mol/L sodium hydroxide TS, add 200 mL of water, neutralize it with 2 mol/L hydrochloric acid, and then add water to make 1 L (hexoprenaline sulfate).

Ascorbic acid See L-ascorbic acid.

Ascorbic acid, for iron test See L-ascorbic acid.

Ascorbic acid for iron test See L-ascorbic acid.

Ascorbic acid-hydrochloric acid TS, 0.2 g/L Dissolve 25 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

Ascorbic acid-hydrochloric acid TS, 0.5 g/L Dissolve 50 mg of L-ascorbic acid in 30 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

Ascorbic acid-sodium iodide TS Dissolve 20 g of L-ascorbic acid and 38.5 g of sodium iodide in water to make 200 mL.

***L*-ascorbic acid** $C_6H_8O_6$ [L(+)-ascorbic acid, Special class]

Aspartic acid See L-aspartic acid.

***L*-aspartic acid** $C_4H_7O_4N$ [Special class]

Aspirin $C_9H_8C_4$ [See monograph, Part II]

Aurintricarboxylic acid ammonium salt See Aluminon.

Balsam Canada balsam for microscopy. Dilute with xylene to an appropriate concentration before use.

Barbital $C_8H_{12}N_2O_3$ [See monograph, Part I].

Barbital buffer solution Dissolve 5.15 g of barbital in 1000 mL of water (A). Add water to 0.75 mL of the hydrochloric acid to make 1000 mL (B). Transfer 50 mL of A solution and 27.5 mL of B solution into a 200-mL volumetric flask, and add water to the gauge line (deoxyribonuclease).

Barbital buffer solution Dissolve 15 g of sodium barbital in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

Barbital buffer solution, pH 8.6 Dissolve 2.21 g of barbital and 12.16 g of sodium barbital in water to make 1000 mL (sodium hyaluronate, sodium hyaluronate ocular injection).

Barbital buffer solution, pH 8.6, ionic strength 0.06 Dissolve 1.62 g of barbital and 12.38 g of sodium barbital in 900 mL of water, adjust the pH to 8.6 with hydrochloric acid, and add water to make 1 L.

Barbital buffer solution, pH 8.6, ionic strength 0.075 Dissolve 2.76 g of barbital and 15.46 g of sodium barbital in 900 mL of water, adjust the pH to 8.6 with hydrochloric acid, and add water to make 1 L.

Barium chloride See barium chloride dihydrate.

Barium chloride dihydrate $BaCl_2 \cdot 2H_2O$ [Special Class]

Barium chloride TS Dissolve 12 g of barium chloride dihydrate in water to make 100 mL (0.5 mol/L).

Barium hydroxide octahydrate $Ba(OH)_2 \cdot 8H_2O$ [Special class] Store in tightly stoppered containers.

Barium hydroxide TS Saturate barium hydroxide octahydrate in freshly boiled and cooled water. Prepare before use (0.25 mol/L).

Barium nitrate $Ba(NO_3)_2$ [Special Class]

Barium nitrate TS Dissolve 6.5 g of barium nitrate in water to make 100 mL (0.25 mol/L).

Barium oxide BaO [For drying].

Barium perchlorate $Ba(ClO_4)_2$ [Special class]

Beclomethasone dipropionate $C_{28}H_{37}ClO_7$ [Special class].

Beeswax [See monograph, Part I onI, Yellow beeswax].

Benzaldehyde C_6H_5CHO [Special class].

Benzaldehyde TS Dissolve 4.0 g of benzaldehyde in 2 mol/L sodium hydroxide to make 100 mL. Prepare before use.

Benzalkonium chloride [See monograph, Part I]

Benzene C_6H_6 [Special class].

Benzene, anhydrous Add sodium to benzene, allow to stand for 24 hours, dehydrate, distill, and collect the oleopten between 79.5 ℃ and 81 ℃.

Benzenesulfonyl chloride $C_6H_5SO_2Cl$ Colorless oily liquid. Insoluble in cold water, and soluble in ethanol and ether. Solidifies at 0 °C.

Melting point: 14 ℃ to 17 ℃
Boiling point: 251℃ to 252 ℃

Benzidine $NH_2C_6H_4C_6H_4NH_2$ [Special class].

Benzidine copper test paper Solution A: To 2 g of benzidine, add 2 g of acetic acid (100) and 100 mL of water, and warmfor 15 minutes to 80 ℃. After cooling, filter under suction. Solution B: Dissolve 3 g of copper(II) acetate in 100 mL of water. Mix 25 mL of Solution A and 2 mL of Solution B. Soak the filter paper in this mixture before use, and use it in a wet state.

Benzidine-copper(II) acetate TS Solution A: Dissolve 1 g of benzidine in 100 mL of dilute acetic acid by warming at 30 ℃ to 40 ℃. Solution B: Dissolve 3 g of copper(II) acetate in 100 mL of water. Mix equal volumes of solution A and B. Prepare before use.

Benzoic acid C_6H_5COOH [Special class].

Benzoin $C_6H_5CH(OH)COC_6H_5$ White to pale yellow, crystals or powder.

Melting point: 132 ℃ to 137 ℃

Benzophenone $C_6H_5COC_6H_5$ Colorless crystals, having a characteristic odor.

Melting point: 48 ℃ to 50 ℃

***p*-Benzoquinone TS** Dissolve 1 g of *p*-benzoquinone in 5 mL of acetic acid (100), and add ethanol to make 100 mL.

***p*-Benzoquinone** $C_6H_4O_2$ Yellow to yellowish brown, crystals or crystalline powder, having a pungent odor. Soluble in ethanol (95) or ether, and slightly soluble in water. It is gradually changed to a blackish brown color by light.

Melting point: 111 ℃ to 116 ℃
Content: NLT 98.0%.

Assay: Weigh accurately about 0.1 g of *p*-benzoquinone, place in an iodine bottle, add exactly 25mL of water and 25 mL of diluted sulfuric acid (1 in 15), dissolve with 3 g of calcium iodide by shaking to mix, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 5.405 mg of $C_6H_4O_2$

Benzoyl chloride C_6H_5COCl A clear, colorless fuming liquid. Density: About 1.2 g/mL

Identification: Determine the infrared absorption spectrum of benzoyl chloride as directed in the liquid film method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1775 cm^{-1}, 1596 cm^{-1}, 1450 cm^{-1}, 1307 cm^{-1}, 1206 cm^{-1}, 873 cm^{-1}, 776 cm^{-1} and 671 cm^{-1}.

***N*-Benzoyl-*L*-isoleucyl-*L*-glutamyl (r-OR)-glycyl-L-arginine-p-nitroanilide hydrochloride** An admixture of compounds with R=H and R=CH_3. It is a white powder.. Sparingly soluble in water.

Absorbance $E_{1cm}^{1\%}$ (316 nm): 166 to 184 [10 mg, water, 300 mL].

Benzyl alcohol $C_6H_5CH_2OH$ A clear, colorless liquid, having a characteristic odor.

Specific gravity d_{20}^{20}: 1.045 to 1.050
Packaging and storage: Preserve in light-resistant, tight containers.

Benzyl benzoate $C_6H_5COOCH_2C_6H_5$ A colorless oily liquid. Congealing temperature: About 18 °C, boiling point: About 323 °C.

Specific gravity d_{20}^{20}: 1.118 to 1.123
Packaging and storage: Preserve in light-resistant, tight containers.

Benzyl ether $(C_6H_5CH_2)_2O$ [Special class]

Benzyl p-hydroxybenzoate $C_{14}H_{12}O_3$ White fine crystals or crystalline powder without odor. Benzyl p-Hydroxybenzoate is freely soluble in ethanol (95) or ether, and very slightly soluble in water.

Melting point: 109 to 112 ℃
Residue on ignition: NMT 0.1%.
Content: NLT 99.0%
Assay: Perform the test as directed under the Assay of Ethyl Paraoxybenzoate in the monograph, Part II.

Each mL/L of 1 mol/L sodium hydroxide VS
= 228.25 mg of $C_{14}H_{12}O_3$

Benzyl p-hydroxybenzoate $HOC_6H_4COOCH_2C_6H_5$ Benzyl p-Hydroxybenzoate is a white fine crystal or crystalline powder, and is odorless. Freely soluble in ethanol, acetone or ether and very slightly soluble in water.

Melting point: 109 to 112℃
Residue on ignition NMT 0.1%.

Assay: Weigh accurately about 1.0 g of benzyl p-hydroxybenzoate, add 20 mL of 1 mol/L sodium hydroxide solution, heat at about 70 ℃ for 1 hour, and then cool immediately. Titrate excess sodium hydroxide with 0.5 mol/L sulfuric acid VS (potentiometric titration under the Titrimetry). The endpoint of the titration is the second inflection point. Perform a blank test in the same way and make necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 228.2 mg of $C_{14}H_{12}O_3$

***p*-Benzylphenol** $C_6H_5CH_2C_6H_4OH$ White to pale yellowish white crystals or crystalline powder.

Melting point: 80 ℃ to 85 ℃

Berberine chloride $C_{20}H_{18}ClNO_4 \cdot \chi H_2O$ [See monograph,

Part I].

BGLB Dissolve 10 g of peptone and 10 g of lactose monohydrate in 500 mL of water, add 200 mL of fresh ox bile or a solution prepared by dissolving 20 g of dried ox bile powder in 200 mL of water and adjusted the pH to 7.0 to 7.5, then add water to make 975 mL, and again adjust pH to 7.4. Then add 13.3 mL of a solution of brilliant green (1 in 1000) and water to make 1000 mL in total volume, and filter through absorbent cotton. Dispense 10 mL volumes of the filtrate into tubes for fermentation, and sterilize by autoclaving at 121 °C for NMT 20 minutes, then cool quickly, or sterilize fractionally on each of three successive days for 30 minutes at 100 °C.

Bilirubin $C_{33}H_{36}N_4O_5$ [Special class]

(S)-BINOL $C_{20}H_{14}O_2$ [Special class, Content: NLT 99%].

2-(4-Biphenyl)propionic acid $C_{15}H_{14}O_2$ Pale yellowish white powder

Melting point: 145 °C to 148 °C

Purity: Dissolve 1 mg of 2-(4-biphenyl)propionic acid in a mixture of water and acetonitrile (11 : 9) to make 50 mL. Perform the test with 20 μL of this solution as directed under the Liquid Chromatography according to the operating conditions Purity (3) Related substances under the Fflubiprofen. Determine each peak area by the automatic integration method at the range of about twice the retention time of the major peak, and determine the amount of 2-(4-biphenyl)propionic acid by the percentage peak area method; NLT 98.0%.

Bis-(1-phenyl-3-methyl-5-pyrazolone) $C_{20}H_{18}N_4O_2$ White to pale yellow crystals or crystalline powder. It dissolves in mineral acids and alkali hydroxide, and it does not dissolve in water, ammonia TS or organic solvents.

Melting point: NLT 300 °C

Residue on ignition: NMT 0.1%.

Nitrogen content: 15.5% to 16.5%

4,4'-Bis(diethylamino)benzophenone $[(C_2H_5)_2NC_6H_4]_2CO$ Pale yellow crystals.

Content: NLT 98%

Assay: Weigh accurately about 0.25 g of 4,4'-bis(diethylamino)benzophenone, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.22 mg of $C_{21}H_{28}N_2O$

Bismuth nitrate See bismuth nitrate pentahydrate.

Bismuth nitrate pentahydrate $Bi(NO_3)_3 \cdot 5H_2O$ [Special Class].

Bismuth nitrate TS Dissolve 5.0 g of bismuth nitrate pentahydrate in acetic acid (100) to make 100 mL.

Bismuth nitrate-potassium iodide TS Dissolve 0.35 g of bismuth nitrate pentahydrate in 4 mL of acetic acid (100) and 16 mL of water, and use this solution as Solution A. Dissolve 8 g of potassium iodide in 20 mL of water and use this solution as the solution B To 20 mL of the mixture of equal volumes of Solutions A and B, add 80 mL of dilute sulfuric acid and 0.2 mL of hydrogen peroxide (30). Prepare before use.

Bismuth potassium iodide TS Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, shake well to mix for 1 hour, add 20 mL of potassium iodide solution (2 in 5), mix, allow to stand for 24 hours, and then filter (Solution A). Dissolve 10 g of L-tartaric acid in 50 mL of water, add 5 mL of Solution *A*, and preserve in a glass-stoppered bottle, protected from the light.

Bismuth subnitrate [See monograph, Part I on Bismuth Subnitrate]

Bismuth subnitrate TS Dissolve 10 g of L-tartaric acid in 40 mL of water. Add 0.85 g of bismuth subnitrate to this solution, shake it for 1 hour for mixing, add 20 mL of a solution of potassium iodide (2 in 5), and shake well for mixing. Allow to stand for 24 hours and filter. Store this solution away from the light.

Bismuth subnitrate-potassium iodide TS, dilute, for spraying Dissolve 10 g of L-tartaric acid in 50 mL. Add 5 mL of bismuth subnitrate TS 5 to this solution.

Bismuth sulfite indicator Prepared for microbial test.

Bistrimethylsilylacetamide $CH_3CON[Si(CH_3)_3]$ Colorless liquid.

Refractive index n_D^{20}: 1.414 to 1.418

Boiling point: 71°C to 73 °C

Specific gravity d_{20}^{20}: 0.825 to 0.835

Blue litmus paper See Litmus paper, blue.

Blue tetrazolium $C_{40}H_{32}Cl_2N_8O_2$ 3,3'-Dianisole-bis- [4,4'-(3,5-diphenyl) tetrazolium chloride]
Pale yellow crystals. Freely soluble in methanol, ethanol (95) or chloroform, slightly soluble in water, and practically insoluble in acetone or ether.

Melting point: About 245 °C (with decomposition)

Absorbance $E_{1cm}^{1\%}$ (252 nm): NLT 826 (methanol).

Blue tetrazolium TS, alkaline Mix 1 volume of a solution of blue tetrazolium in methanol (1 in 200) and 3 volumes of a solution of sodium hydroxide in methanol (3 in 25). Prepare before use.

Borane-pyridine complex C_5H_8BN Content NLT 80%.

Assay: Weigh accurately about 30 mg of borane-pyridine complex, dissolve in 40 mL of 0.05 mol/L iodine solution, add 10 mL of diluted sulfuric acid (1 in 6), and titrate the resulting solution with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank titration in the same manner to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.549 mg of C_5H_8BN

Borate buffer solution Dissolve 2.5 g of sodium chloride, 2.85 g of borax and 10.5 g of boric acid in water to make 1000 mL. At this time, the pH is 7.5 ± 0.1 (preserve: 0 °C ice water) (pancreazeI, pancreazeII).

Borate buffer solution, methanolic Dissolve 21 g of bo-

ric acid in 280 mL of 1 mol/L sodium hydroxide TS and add water to make 1 L. To 50 mL of this solution, add methanol, shake to mix, cool, and add methanol to make 100 mL.

Borate buffer solution, pH 13.0 Dissolve 5.0 g of boric acid and 6.0 g of potassium chloride in water, add 300 mL of 0.5 mol/L sodium hydroxide TS, and add water to make 1 L.

Borate buffer solution, pH 7.0 To 39 mL of 0.05 mol/L sodium borate TS, add 61 mL of 0.2 mol/L potassium dihydrogen phosphate.

Borate buffer solution, pH 8.2 Dissolve 19.1 g of borax in water to make 1 L (0.2 mol/L). To this solution, add 0.1 mol/L hydrochloric acid to adjust the pH to 8.2.

Borate buffer solution, pH 9.0 Dissolve 12.36 g of boric acid and 16.9 g of potassium chloride in water, add 300 mL of 0.5 mol/L sodium hydroxide TS, and add water to make 1 L.

Borate-hydrochloric acid buffer solution, pH 9.0 Dissolve 19 g of sodium tetraborate decahydrate in 900 mL of water, adjust the pH to 9.0 with 1 mol/L hydrochloric acid, and add water to make 1 L (serratiopeptidase).

Borax See sodium tetraborate decahydrate.

Boric acid H_3BO_3 [Special class]

Boric acid buffer solution, pH 7.5 Dissolve 2.5 g of sodium chloride and 2.85 g of borax in water to make 100 mL. Adjust the pH to 7.5±0.1 with 0.1 mol/L hydrochloric acid TS or 0.1 mol/L sodium hydroxide TS (preserve: 0 ℃ ice water) (pancreatin).

Boric acid ethanol TS To 20 g of boric acid, add ethanol (99.5), dissolve by warming, cool, and add ethanol (99.5) to make 1 L.

Boric acid solution Dissolve 4.95 g of boric acid in 50 mL of water, adjust the pH to 9.1 with 1 mol/L potassium hydroxide TS, and add water to make 100 mL (sodium hyaluronate, sodium hyaluronate ocular injection).

0.2 mol/L Boric acid-0.2 mol/L potassium chloride TS for buffer solution See 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS, for buffer solution.

0.2 mol/L boric acid-0.2 mol/L potassium chloride TS, for buffer solution Dissolve 12.376 g of boric acid for buffer solution and 14.911 g of potassium chloride in water to make 1000 mL.

Boric acid-methanol buffer solution Weigh accurately 2.1 g of boric acid, dissolve in 28 mL of sodium hydroxide TS, and add water to make accurately 100 mL. Mix 1 volume of this solution and 1 volume of methanol by shaking.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0 Dissolve 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride for buffer solution and 43.90 mL of 0.2 mol/L sodium hydroxide in water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 13.0 Dissolve 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution in 0.2 mol/L sodium hydroxide to make the pH to 13.0. Preserve in polyethylene bottles (bisbentiamine).

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0 Dissolve 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution and 21.30 mL of 0.2 mol/L sodium hydroxide in water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.2 Dissolve 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution and 26.70 mL of 0.2 mol/L sodium hydroxide in water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 Dissolve 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride for buffer solution and 36.85 mL of 0.2 mol/L sodium hydroxide in water to make 200 mL.

Boric acid-sodium hydroxide buffer solution, pH 10.0 Dissolve 29.6 g of boric acid in 700 mL of water, adjust the pH to 10.0 with sodium hydroxide (3 in 10), and add water to make 1 L.

Boric acid-sodium hydroxide buffer solution, pH 11.0 Mix equal amounts of 0.2 mol/L sodium borate TS and 0.1 mol/L sodium hydroxide TS (hymecromone).

Boric acid-sodium hydroxide buffer solution, pH 8.4 Dissolve 24.736 g of boric acid in 0.1 mol/L sodium hydroxide VS to make exactly 1000 mL.

Boron trifluoride BF_3 Colorless gas, having an irritating odor.

Melting point: About -127.1 ℃
Boiling point: -100.3 ℃

Boron trifluoride-methanol TS A methanol solution containing 14 g/dL of boron trifluoride (BF_3 : 67.81).

Bovine coagulation factor X A protein obtained from bovine serum that selectively degrades prothrombin to thrombin. It does not contain thrombin or plasmin. Bovine coagulation factor X contains NLT 500 units per mg. One unit of Bovine coagulation factor X is the amount of protein that hydrolyzes 1 μmol of N-benzoyl-L-isoleucyl-L-glutamyl (*r*-OR)-glycyl-L-arginine-*p*-nitroanilide at 25 °C for 1 minute.

Bovine serum albumin Obtained from bovine serum as Cohn's fifth fraction and contains NLT 95% of albumin.

Bovine serum albumin-isotonic sodium chloride injection Dissolve 0.1 g of Bovine serum albumin in 100 mL of Isotonic sodium chloride injection . Prepare before use.

Brij 35 To prepare Brij 35, perform addition polymerization with with 1 mole of lauryl alcohol and 23 mole of ethylene oxide to obtain polyoxyethylene ether. A white solid with a characteristic odor (sodium chondroitin sulfate).

Brilliant green $C_{27}H_{34}N_2O_4S$ Fine, glistening, yellow crystals. It dissolves in water and ethanol (95).

The wavelength of absorption maximum: 623 nm.

Bromine Br [Special class].

Bromine solution, 0.5 mol/L 1 L of this solution contains 39.955 g of bromine (Br: 79.91).

Preparation: Dissolve 14 g of potassium bromate and 60 g of potassium bromide in water to 1 L, and standardize as follows.

Standardization: Transfer exactly 25 mL of the previously prepared bromine solution into an iodine flask, add 120 mL of water and 5 mL of hydrochloric acid, stopper the flask, and shake gently to mix. Then add 5 mL of potassium iodide TS, stopper the bottle again, and shake gently to mix. Allow to stand for 5 minutes, titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS, and determine the factor. However, when the solution becomes a pale yellow color at around the endpoint, add 3mL of starch TS; the solution exhibits a blue color. Continue the titration until the blue color disappears. Perform a blank test in the same manner and make any necessary correction.

Bromine TS Prepare by saturating bromine in water. Transfer 2 mL - 3 mL of bromine to a bottel with a glass stopper which has been applied with vaseline, add 100 mL of cold water, cover the stopper, and shake to mix. Preserve preferably in a cold place, protected from light.

Bromine-acetic acid TS Dissolve 10 g of sodium acetate trihydrate in acetic acid (100) to make 100 mL, add 5 mL of bromine, and shake to mix. Preserve preferably in a cold place, protected from light.

Bromine-carbon tetrachloride TS To 0.1 g of bromine, add carbon tetrachloride to make 100 mL. To 2 mL of this solution, add carbon tetrachloride to make 10 mL. Prepare before use.

Bromine-sodium hydroxide TS To 100 mL of sodium hydroxide (3 in 100), add 0.2 mL of bromine. Prepare before use.

Bromocresol green $C_{21}H_{14}Br_4O_5S$ [Special class].

Color change interval: pH 3.8 (yellow) to 5.4 (blue)

Bromocresol green TS Dissolve 50 mg of bromocresol green in 100 mL of ethanol (95). Filter, if necessary.

Bromocresol green TS Weigh accurately 69.8 mg of bromocresol green, dissolve in 2 mL of 0.1 mol/L sodium hydroxide TS, and add water to make exactly 1 L (diphenhydramine hydrochloride).

Bromocresol green TS (1 in 1000) To 100 mg of bromocresol green, add 8.0 mL of 0.02 mol/L sodium hydroxide TS, soften, and add water to make 100 mL. Filter if necessary (ephedrine hydrochloride)

Bromocresol green-methyl red TS Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in 180 mL of ethanol (99.5), and add water to make 200 mL.

Bromocresol green-methylrosaniline chloride Dissolve 0.3 g of bromocresol green and 75 mg of methylrosaniline chloride in 2 mL of ethanol (95), and add acetone to make 100 mL.

Bromocresol green-sodium hydroxide TS To 0.2 g of bromocresol green, add 2.8 mL of 0.1 mol/L sodium hydroxide, soften in a mortar, and add water to make 200 mL. Filter, if necessary.

Bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS To 0.25 g of bromocresol green, add 15 mL of water and 15 mL of dilute sodium hydroxide TS, dissolve with a small quantity of acetic acid-sodium acetate buffer solution, pH 4.5, while shaking, and add acetic acid-sodium acetate buffer solution, pH 4.5, to make 500 mL. Wash 250 mL of this solution twice with 100 mL of dichloromethane. Filter, if necessary.

Bromocresol green-sodium hydroxide-ethanol TS Dissolve 50 mg of bromocresol green in 0.72 mL of 0.1 mol/L sodium hydroxide and 20 mL ethanol, and add water to make 100 mL.

Purity: To 0.2 mL of this test solution, add 100 mL of freshly boiled and cooled water; the color of the solution is blue. To this solution, add 0.02 mol/L hydrochloric acid TS until the color of the solution is changed to yellow; NMT 0.2 mL is consumed.

Bromocresol purple $C_{21}H_{16}Br_2O_5S$ [Special class].

Bromocresol purple TS Dissolve 50 mg of bromocresol purple in 100 mL of ethanol (95). Filter, if necessary.

Bromocresol green TS Dissolve 50 mg of bromocresol purple in sodium monohydrogen phosphate-citric acid buffer solution to make 100 mL (pridinol mesilate).

Bromocresol purple-McIlvaine buffer solution To 37.5 mg of bromocresol purple, add mcIlvaine buffer solution, pH 6.0, to make 100 mL.

Bromocresol purple-potassium monohydrogen phosphate-citric acid TS Mix 30 mL of bromocresol purple-sodium hydroxide TS and 30 mL of dibasic potassium phosphate-citric acid buffer solution, pH 5.3, and wash three times with 60 mL of chloroform.

Bromocresol purple-sodium hydroxide TS To 0.4 g of bromocresol purple, add 6.3 mL of dilute sodium hydroxide TS, soften in a mortar, and add water to make 250 mL. Filter, if necessary.

Bromocyclohexane TS Dissolve 0.1 g of bromine in cyclohexane to make 100 mL. To 2 mL of this solution, add cyclohexane to make 10 mL. Prepare before use.

Bromophenol blue $C_{19}H_{10}Br_4O_5S$ [Special class].

Bromophenol blue solution Dissolve 40 mg of bromophenol blue in 100 mL of water, and add 1 mL of 0.1 mol/L sodium hydroxide (bezenthonium chloride).

Bromophenol blue TS Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol. Filter, if necessary.

Bromophenol blue TS-potassium hydrogen phthalate TS Dissolve 0.1 g of bromophenol blue in potassium hydrogen phthalate buffer solution, pH 4.6, to make 100 mL.

Bromophenol blue TS, dilute Dissolve 50 mg of bromophenol blue in 100 mL of ethanol (99.5). Prepare before use.

Bromophenol blue TS, pH 7.0 Mix 10 mL of bromophenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with diluted sodium hydroxide TS (1 in 10).

Bromophenol blue-potassium hydrogen phthalate TS To

0.1 g of bromophenol blue, add potassium hydrogen phthalate buffer solution, pH 4.6, to make 100 mL (hydrocortisone acetate-allantoin-diphenhydramine hydrochloride ointment).

***N*-Bromosuccinimide** $C_4H_4BrNO_2$ [Special class].

***N*-Bromosuccinimide TS** Dissolve 1 g of N-bromosuccinimide in 1000 mL of water.

Bromothymol blue $C_{27}H_{28}Br_2O_5S$ [Special class].

Bromothymol blue solution, 0.01 mol/L Place 0.625 g of sodium salt of bromothymol blue in a 100 mL volumetric flask, dissolve with 10 mL of 0.1 mol/L hydrochloric acid, and add water to make 1 L (expiration date: 1 month) (emepronium bromide).

Bromothymol blue TS See bromothymol blue TS.

Bromothymol blue TS Dissolve 0.1 g of bromothymol blue in 100 mL of dilute ethanol. Filter, if necessary.

Bromothymol blue TS Dissolve 45 mg of bromothymol blue in phosphate buffer solution, pH 7.6, to make 250 mL, transfer to a separatory funnel, and extract three times with 15 mL of chloroform. Discard the chloroform layer, and use the aqueous layer (scopolamine bromide).

Bromothymol blue TS Dissolve 150 mg of bromothymol blue and 150 mg of anhydrous sodium carbonate in water to make 100 mL, and filter. Prepare this test solution just before use.

Bromothymol blue TS, 0.1% Dissolve 100 mg of bromothymol blue in 80 mL of 0.02 mol/L sodium hydroxide TS hydroxide VS by softening, and add water to make 100 mL. Filter, if necessary.

Bromothymol blue-neutral red TS Dissolve 0.2 g of bromothymol blue and 0.2 g of neutral red in ethanol to make 100 mL (lactobacillus sporogenes).

Bromothymol blue-sodium hydroxide TS To about 0.2 g of powdered bromothymol blue, add 5mL of dilute sodium hydroxide TS, dissolve with a small quantity of water by shaking to mix on a water bath at 50 ℃, and add water to make 100 mL.

Bromovalerylurea $C_6H_{11}BrN_2O_2$

Broth, normal See normal broth.

Brucin See brucin dihydrate.

Brucine dihydrate $C_{23}H_{26}N_2O_4 \cdot 2H_2O$ [Special class].

Buffer solution for lipase suspension Dissolve 10 g of sodium chloride, 6.06 g of trishydroxymethylaminoethane and 4.90 g of malic acid anhydrous in 900 mL of water, adjust its pH to 7 with 4 mol/L sodium hydroxide solution, and add water to make 1000 mL.

Buffer solution, for free protease test Dissolve 2.5 g of sodium chloride, 10.5 g of boric acid, and 2.85 g of disodium tetraborate decahydrate in 900 mL of water to adjust the pH to 7.5 ± 1, and add water to make 1000 mL (refrigerate) (pancrease).

1-Butanol, ammonia saturated To 100 mL of 1-butanol, add 60 mL of dilute ammonia water (28) (1 in 100), shake vigorously for 10 minutes, and then allow to stand. Use the upper layer.

Butanol, iso See 2-methyl-1-propanol.

Butanol, sec See 2-butanol.

Butanol, tert See *t*-butyl alcohol

1-Butanol $CH_3(CH_2)_2CH_2OH$ [Special class]

2-Butanol $CH_3CH_2CH(OH)CH_3$ [Special class].

***n*-Butanol** See 1-butanol.

***tert*-Butanol** See t-Butyl alcohol.

2-Butanone $CH_3COC_2H_5$ [Special class].

Butyl acetate n-butyl acetate

***n*-Butyl acetate** $CH_3COOCH_2CH_2CH_2CH_3$ [Special class]

***t*-Butyl alcohol** $(CH_3)_3COH$ [Special class]

***n*-Butyl chloride** See 1-chlorobutane

***n*-Butyl formate** $HCOO(CH_2)_3CH_3$ A clear and colorless liquid, having a characteristic odor.
Specific gravity d_{20}^{20}: 0.884 to 0.904

Butyl p-hydroxybenzoate $HOC_6H_4COOCH_2CH_2CH_2CH_3$ [See monograph, Part II]

***n*-Butylamine** $CH_3CH_2CH_2CH_2NH_2$ A colorless liquid, having an amine-like, characteristic odor. Miscible with water, ethanol or ether. The aqueous solution shows alkalinity and easily absorbs carbon dioxide from the air.
Specific gravity d_{20}^{20}: 0.740 to 0.747
Distilling range: 76.5 ℃ to 79 ℃, NLT 96 vol%.

4-(Butylamino)benzoic acid $C_{11}H_{15}NO_2$ [Special class]

***tert*-butylmethylether** $(CH_3)_3COCH_3$ Colorless transparent liquid, having a specific odor.
Refractive index n_D^{20}: 1.3689
Specific gravity d_{20}^{20}: 0.7404

Butyrolactone $C_4H_6O_2$ Clear, colorless to practically colorless transparent liquid.
Boiling point: 198℃ to 208 ℃
Specific gravity d_{20}^{20}: 1.128 to 1.135

Cadmium acetate See cadmium acetate dihydrate.

Cadmium acetate dihydrate $Cd(CH_3COO)_2 \cdot 2H_2O$ $Cd(CH_3COO)_2 \cdot 2H_2O$ White crystals or crystalline powder.
Identification (1) Dissolve 0.2 g of cadmium acetate dihydrate in 20 mL of water, and use this as the test solution. To 10 mL of this solution, add 2 mL of iron (III) chloride TS; a reddish brown color is produced.
(2) To 10 mL of the test solution obtained in (1), add 1 mL of sodium sulfide TS; a yellow precipitate is produced.

Cadmium, crude metal Cd [First Class]

Cadmium-ninhydrin TS Dissolve 50 mg of cadmium acetate dihydrate in 5 mL of water and 1 mL of acetic acid (100), add 2-butanone to make 50 mL, and dissolve 100 mg of ninhydrin in this solution. Prepare before use.

Cadmium, crude metal Cd [First Class]

Caffeine $C_8H_{10}N_4O_2 \cdot H_2O$ [See monograph, Part I on Caffeine Hydrate]

Caffeine, anhydrous $C_8H_{10}N_4O_2$ [See monograph, Part I on Anhydrous Caffeine]

Calcium acetate See calcium acetate hydrate.

Calcium acetate hydrate CH_3COOCa [Special class]

Calcium carbonate $CaCO_3$ [Calcium carbonate, Special Class]

Calcium chloride See calcium chloride dihydrate.

Calcium chloride dihydrate $CaCl_2 \cdot 2H_2O$ [Special Class]

Calcium chloride for drying See calcium chloride, for drying.

Calcium chloride for Karl Fischer titration See calcium chloride, for Karl Fischer titration.

Calcium chloride solution Dissolve 294 g of calcium chloride in 300 mL of water, adjust the pH to 6.0 to 6.2 with 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide solution, and add water to make 1000 mL (store at 0 ℃ to 4 ℃).

Calcium chloride solution, 0.1 mol/L Dissolve 1 g of calcium chloride in water to make 1000 mL (diastase and protease 500).

Calcium chloride TS Dissolve 7.5 g of calcium chloride dihydrate in water to make 100 mL (0.5 mol/L).

Calcium chloride, for drying $CaCl_2$ [For drying]

Calcium chloride, for Karl Fischer titration $CaCl_2$ [For Karl Fischer titration]

Calcium hydroxide $Ca(OH)_2$ [First class].

Calcium hydroxide for pH measurement See calcium hydroxide, for pH measurement.

Calcium hydroxide TS To 3 g of calcium hydroxide, add 1000 mL of cold distilled water, and occasionally shake the mixture for 1 hour to mix. Allow it to stand and use the supernatant (0.04 mol/L).

Calcium hydroxide, for pH measurement Calcium hydroxide prepared for pH measurement.

Calcium hydroxide, pH standard solution See the pH Measurement under the General Tests.

Calcium nitrate See calcium nitrate tetrahydrate.

Calcium nitrate tetrahydrate $Co(NO_3)_2 \cdot 4H_2O$ [Special Class]

Calcium oxide CaO [Calcium oxide (burnt lime) first class]

Calcium tocopherol succinate $C_{66}H_{106}CaO_{10}$ [See monograph, Part I]

Camphor $C_{10}H_{16}O$ [See monograph, Part I on *d*-Camphor or *dl*-Camphor]

***d*-Camphorsulfonic acid** $C_{10}H_{16}O_4S$ White crystals or crystalline powder, having a characteristic odor. Freely soluble in water, and soluble in chloroform.

Purity *Clarity and color of solution*: Dissolve 1.0 g of *d*-camphorsulfonic acid in 10 mL of water: the solution is clear, colorless to pale yellow.

Loss on drying: NMT 2.0% (1 g, 105 ℃, 5 hours).

Content: NLT 99.0%, calculated on the dried basis.

Assay: Dissolve about 4 g of *d*-camphorsulfonic acid, accurately weighed, with 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 232.30 mg of $C_{10}H_{16}O_4S$

Capric acid $C_{10}H_{20}O_2$ White powder. Soluble in ethanol, chloroform and ether, and practically insoluble in water.

Melting point: 30 ℃ to 33 ℃

Carbazochrome $C_{10}H_{12}N_4O_3$ Yellowish red to red crystals or crystalline powder.

Melting point: About 222 ℃ (with decomposition)

Content: NLT 98.0%.

Assay: Dissolve about 0.2 g of carbazochrome, weighed accurately, in 20 mL of acetic acid (100) by warming, add 80 mL of acetic anhydride, cool, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.623 mg of $C_{10}H_{12}N_4O_3$

Carbazole $C_{12}H_9N$ White foliaceous or plate-like crystals or crystalline powder. Freely soluble in pyridine and in acetone, slightly soluble in ethanol, and practically insoluble in water. It readily sublimes when heated.

Melting point 243 ℃ to 245 ℃

Purity *Clarity and color of solution:* To 0.5 g of Carbazole, add 20 mL of ethanol, warm it to dissolve: the solution is clear.

Residue on ignition: NMT 0.1%.

Carbon dioxide CO_2 [See monograph, Part I]

Carbon disulfide CS_2 [Special Class] Preserve in a stoppered container in a cold and dark place away from fire.

Carbon disulfide TS Mix 35 mL of carbon disulfide TS, 55 mL of pyridine and 65 mL of isopropyl alcohol (diethanolamine glucuronate, betaine glucuronate and nicotinamide ascorbate injection).

Carbon monoxide CO A toxic, colorless gas. Prepare by passing the gas evolved by reacting formic acid with sulfuric acid through a layer of sodium hydroxide TS.

Carbon monoxide detector column Melt-sealed glass column designed so that an appropriate adsorption filter and gas containing iodine pentoxide (indicator), selenium dioxide and fuming sulfuric acid, for detection of carbon monoxide, can pass through (carbon monoxide determination range between 5 and 150 ppm)

Carbon tetrachloride CCl_4 [Special class].

Carbonate buffer solution, pH 10.2

0.2 mol/L sodium carbonate solution (Solution A): Dissolve 21.2 g of sodium carbonate in water to make 1 L.

0.2 mol/L sodium carbonate solution (Solution B): Dissolve 16.8 g of sodium bicarbonate in water to make 1 L.

Mix 150 mL of Solution A and 10 mL of Solution B, add water to make 1 L, and adjust the pH to 10.2 by adding small volumes of Solution A and Solution B (emepronium bromide).

Carmofur $C_{11}H_{15}FN_3O_3$ [See monograph, Part I]

Casein (milk) [Special Class]

Casein peptone See casein peptone.

Casein solution To about 0.6 g of milk casein, previously dried at 105 ℃ for 2 hours and accurately weighed, add 6 mL of a 1000 mL lactic acid TS prepared by mixing lactic acid 120 g and water, and add 75 mL of water, heat the mixture on a water bath at 60 to 70 ℃ for 15 minutes to dissolve, cool it with water, adjust the pH to 3.0 with 1 mol/L hydrochloric acid TS or 1 mol/L sodium hydroxide TS, and then add water to make 100 mL (newlase).

Casein solution, 0.6%, pH 2.0 Add 5 mL of 0.1 mol/L hydrochloric acid to 0.6 g of milk casein, allow to stand for 15 minutes, slowly stir it on a water bath, and add water to make 40 mL. After cooling, add 50 mL of 0.1 mol/L acetate buffer solution (pH 2.0), adjust the pH to 2.0 with 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium acetate solution, and add water to make 100 mL (gastropylore powder).

Casein solution, 0.6%, pH 7.2 Add 20 mL of 0.1 mol/L sodium hydroxide solution to about 0.6 g of milk casein, previously dried, accurately weighed, heat it to dissolve, cool it, adjust the pH to 7.2 with0.1 mol/L phosphoric acid, and add water to make 100 mL (diastase and protease 100, diastase and protease 500).

Casein solution, pH 6.0 Dry about 1 g of milk casein at 105 ℃ for 2 hours, measure the loss in mass, accurately weigh the amount equivalent to 0.6 g as a dried material, add 0.05 mol/L sodium monohydrogen phosphate TS 80 mL to it, and heat it on a water bath for dissolution. After cooling, adjust the pH to 6.0 with 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide solution, and add water to make 100 mL. Prepare before use (Diastase·Protease N1).

Casein solution, pH 8.0 Place 1. 25 g of casein (Merck No.2244) in a 100-mL beaker, add 5.0 mL of water and stir with a glass rod. Add 10.0 mL of 0.1 mol/L sodium hydroxide TS, mix, and add 60 mL of water and stir continuously with a magnetic stirrer until casein dissolves completely. Adjust the pH to 8.0 with 0.1 mol/L hydrochloric acid TS or 0.1 mol/L sodium hydroxide TS, transfer it to a 100-mL volumetric flask, and add water to the gauge line (use within 24 hours after preparation) (pancreatin).

Casein solution, pH 9.0 Determine loss on drying of casein, previously dried for 3 hours at 60 ℃, in vacuum (NMT 0.67 kpa). Then, weigh accurately the amount of casein equivalent to 1.2 g, dried, add 160 mL of sodium borate solution (19 in 1000), and heat it on a water bath to dissolve. After cooling, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid, and add borate-hydrochloric acid buffer solution (pH 9.0) to make 200.0 mL. Warm the mixture at 37 ± 0.5 ℃. Prepare before use (serratiopeptidase).

Casein substrate solution, 0.6% Dissolve 0.6 g of milk casein in 80 mL of 0.05 mol/L sodium monohydrogen phosphate TS, adjust the pH to 7.0 with 1 mol/L hydrochloric acid, and add water to make 100 mL. Prepare before use (bromelain).

Casein TS, pH 8.0 Add 100 mL of pH 8.0 phosphate buffer solution to 1.20 g of milk casein, dried and accurately weighed, heat it at 65 to 70 ℃ for 15 minutes to dissolve, cool it, adjust the pH to 8.0 with sodium hydroxide TS, and add water to make 200 mL (semi-alkaline protease).

Castor oil [See monograph, Part II].

Catechol $C_6H_4(OH)_2$ [Catechol (pyrocatechin), Special Class]

Cefdinir lactam ring-cleavage lactones $C_{14}H_{15}N_5O_6S_2$ White to yellow powder, a mixture of 4 diastereomers.

Identification: Determine the infrared spectra of Cefdinir lactam ring-cleavage lactones as directed in the paste method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1743 cm^{-1}, 1330 cm^{-1}, 1163 cm^{-1}, and 1047 cm^{-1}.

Content: NLT 90%.

Assay: Weigh accurately about 5 mg of cefdinir lactam ring-cleavage lactones, dissolve in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the test solution. Perform the test with 5 μL of this solution as directed under the Purity (2) Related substances under Cefdinir, and calculate the areas of each peak of the test solution by the automatic integration method. Determine the ratio of the total peak areas of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

Celite column Add about 200 g of celite545 in 2 L of dilute hydrochloric acid, boil by shaking for 10 minutes, wash with water until the washings become netural on addition of methyl red, and dry at 105 ℃ for 15 minutes. To 15 g of the dried residue, add 5 mL of 0.5 mol/L phosphate buffer solution. Pack this solution into a chromatography column (15× 350mm).

Celite, for test To about 500 g of celite545, add a sufficient amount of hydrochloric acid, stir, allow to stand for 24 hours, and filter. Wash with water until the washings becomes neutral, wash with 200 mL of methanol, wash again with 200 mL of ether, air-dry, dry completely at 100 ℃.

Cellulose for thin layer chromatography A high quality cellulose prepared for thin layer chromatography.

Cellulose for thin layer chromatography (with fluorescent indicator) Cellulose for thin layer chromatography containing fluorescent indicator.

Cellulose tris-(3,5-dimethylphenylcarbamate) for liquid chromatography Prepared for coated porous silica gel for Liquid chromatograph.

Cephaeline hydrobromide $C_{28}H_{38}N_2O_4 \cdot 2HBr \cdot \chi H_2O$ White or pale yellow crystalline powder.

Purity: Dissolve 10 mg of Cephaeline hydrobromide in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under the Liquid Chromatography of the Assay in Ipecac; the area of any peak other than the peak of cephaeline obtained from the test solution is smaller than the peak area of cephaeline obtained from the standard solution

Cerium sulfate $Ce(SO_4)_2$ [Special class]

Cerium sulfate solution, 0.1 mol/L 1000 mL of cerium sulfate solution contains 33.2 g of cerium sulfate [$Ce(SO_4)_2$: 332.24].

Preparation: To 59 g of cerium(IV) diammonium nitrate, add 31 mL of sulfuric acid, carefully add 20 mL of water until dissolved, allow to stand for 24 hours, filter through a glass filter, add water again to make 1000 mL, and standardize the solution as follows:

Standardization: Weigh 0.2 g of arsenic trioxide, previously dried at 105 °C for 1 hour, and place in a 500-mL Erlenmeyer flask. Wash the inner wall with 25 mL of sodium hydroxide solution (2 in 25), dissolve while stirring, add 10 mL of water, mix, and add diluted sulfuric acid (1 in 3). Add 2 drops of o-phenanthrolein TS and 2 drops of 0.05 mol/L sulfuric acid solution of osmium tetroxide (1 in 400), and titrate with the prepared 0.1 mol/L cerium sulfate VS. However, the endpoint of the titration is when the color of the solution changes from pink to pale blue.

Each mL of 0.1 mol/L cerium sulfate VS
4.946 mg of AS_2O_3

Cerium sulfate-ammonium molybdate TS Add diluted sulfuric acid (3 in 50) to 1 g of cerium sulfate tetrahydrate and 2.5 g of ammonium heptamolybdate(VI) tetrahydrate, and dissolve to make 100 mL. Prepare before use.

Cerium-alizarin complexone TS To 30 mL of acetone, add 68 mL of acetate buffer solution, pH 4.0, 10 mL of alizarin complexone TS and 10 mL of 0.7% cerium nitrate, and add water to make 500 mL. Prepare before use.

Cerous nitrate See cerium (III) nitrate hexahydrate.

Cerium(III) nitrate hexahydrate $Ce(NO_3)_3 \cdot 6H_2O$ Colorless to pale yellow, crystalline powder. It is soluble in water. Chloride: NMT 0.036%, sulfate: NMT 0.12%.

Content: NLT 98.0%.

Assay: To about 1.5 g of Cerium (III) nitrate hexahydrate, accurately weighed, add 5 mL of sulfuric acid, and heat until white fumes are evolved vigorously. After cooling, add 200 mL of water, 0.5 mL of 0.1 mol/L silver nitrate solution, and 5 g of ammonium persulfate to dissolve, and boil the mixture for 15 minutes. After cooling, add 2 drops of o-phenanthroline TS, and titrate with 0.1 mol/L ammonium iron (II) sulfate solution until the pale blue color of the solution changes to red.

Each mL of 0.1 mol/L ferrous ammonium sulfate VS
= 43.42 mg of $Ce(NO_3)_3 \cdot 6H_2O$

Cerium(III) nitrate TS Dissolve 0.44 g of cerium(III) nitrate hexahydrate in water to make 1000 mL.

Cerium(IV) diammonium nitrate $Ce(NH_4)_2(NO_3)_6$ [Special Class]

Cerium(IV) diammonium nitrate TS Dissolve 6.25 g of cerium (IV) diammonium nitrate in 160 mL of diluted dilute nitric acid (9 in 50). Use within 3 days after preparation.

Cerous nitrate See cerium (III) nitrate hexahydrate.

Cerous nitrate TS See cerium (III) nitrate TS.

Cetanol [See monograph, Part II].

Cetrimide $C_{17}H_{38}BrN$ White to pale yellowish white powder, having a slightly characteristic odor.

Purity *Clarity and color of solution:* Dissolve 1.0 g of cetrimide in 5 mL of water; the solution is clear.

Content: NLT 96.0%.

Assay: Weigh accurately about 2 g of cetrimide, previously dried, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution into a separator, add 25 mL of chloroform, 10 mL of 0.1 mol/L sodium hydroxide and 10 mL of freshly prepared potassium iodide (1 in 20), shake vigorously to mix, allow to stand, and remove the chloroform layer. Wash three times with 10 mL of chloroform, take the water layer, and add 40 mL of hydrochloric acid. After cooling, titrate with 0.05 mol/L potassium iodide VS until the color of the solution almost disappears. Add 2 mL of chloroform again, and titrate until the redish purple color of the chloroform layer disappears. The endpoint is when the redish purple color of the chloroform layer no more reappears within 5 minutes after the chloroform layer is decolorized. Perform a blank test with 20 mL of water, 10 mL of a solution of potassium iodide (1 in 20) and 40 mL of hydrochloric acid.

Each mL of 0.05 mol/L potassium iodate VS
= 33.640 mg of $C_{17}H_{38}BrN$

Chalcone-carboxylic acid $C_{21}H_{14}N_2O_7S$

Chalcone-carboxylic acid Indicator Grind 200 mg of chalcone-carboxylic acid and 20 g of potassium sulfate for mixing.

Chenodeoxycholic acid $C_{24}H_{40}O_4$ White crystals or crystalline powder.

Very soluble in methanol or acetic acid (100), freely soluble in ethanol (95), soluble in acetone, sparingly soluble in ethyl acetate, slightly soluble in chloroform and practically insoluble in water.

Melting point: About 119 °C (ethyl acetate recrystalization)

Purity *Related substances*: Dissolve 25 mg of Chenodeoxycholic acid in a mixture of chloroform and ethanol (9 : 1) to make exactly 250 mL. Perform the test with 10 μL of this solution as directed in the Purity (7) under Ursodeoxycholic Acid; no spot other than the principal spot with the R_f value of about 0.4 appears.

Content: NLT 98.0%

Assay: Weigh accurately about 0.5 g of Chenodeoxycholic acid, previously dried in vacuum for 4 hours at 80 °C, and add 40 mL of neutralized ethanol and 20 mL of water to dissolve. Then,

add 2 drops of phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS, and toward the endpoint of titration, add 100 mL of freshly boiled and cooled water and titrate again.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.258 $C_{24}H_{40}O_4$

Chiral-recognition protein ovomucoid-linked silica gel for liquid chromatography Prepared for liquid chromatography.

Chloral hydrate $CCl_3CH(OH)_2$ [First class]

Chloral hydrate TS Dissolve 5 g of chloral hydrate in 3 mL of water.

Chloramine $C_7H_7ClNNaO_2S\cdot 3H_2O$ [Chloramine T, Special Class]

Chloramine TS Dissolve 1 g of chloramine in water to make 100 mL. Prepare before use.

Chloramphenicol $C_{11}H_{12}C_{12}N_2O_5$ [See monograph, Part I]

Chlordiazepoxide $C_{16}H_{14}ClN_3O$ [See monograph, Part I]

Chlorinated lime [See monograph, Part II]

Chlorinated lime TS Add 9 mL of water to 1 g of chlorinated lime, grind, mix, and filter. Prepare before use.

Chlorine Cl_2 A yellowish green gas, having a suffocating odor. It is heavier than air, and is soluble in water. It is prepared by reacting chlorinated lime with hydrochloric acid. Chlorine from a metal cylinder may also be used.

Chlorine TS Use a saturated aqueous solution of chlorine. Preserve this solution fully filled in light-resistant, glass-stoppered bottles, preferably in a cold place.

3-Chloro-1,2-propanediol $C_3H_7ClO_2$ [First Class]

3-chloro-2-methylaniline C_7H_8ClN [First Class]

1-chloro-2,4-dinitrobenzene $C_6H_3(NO_2)_2Cl$ [Special Class]

4-chloroaniline $H_2NC_6H_4Cl$ White crystals or crystalline powder. Freely soluble in ethanol or acetone, and soluble in hot water.

Melting point: 70 to 72 ℃
Residue on ignition: NMT 0.1% (1 g).

***p*-Chloroaniline** See 4-chloroaniline.

Chloroauric acid See hydrogen tetrachloroaurate(III) tetrahydrate.

Chloroauric acid TS See tetrachloroaurate TS.

Chlorobenzene C_6H_5CI Clear, colorless liquid, having a characteristic odor. Insoluble in water, and soluble in ethanol, benzene, chloroform and ether.

Specific gravity:1.100 to 1.111
Boiling point: NLT 95% evaporates at 129 to 131 ℃.
Acidity: Add methyl red TS to 200 mL of methanol and neutralize it with 0.1 mol/L sodium hydroxide solution (ignore the consumed amount at this point). Dissolve 23 mL of Chlorobenzene in neutralized methanol and titrate it with 0.1 mol/L sodium hydroxide VS: the consumed amount is NMT 1.0 mL (as 0.015% hydrochloric acid).

Residue on evaporation: Evaporate 91 mL of Chlorobenzene, and dry at 105 ℃ for 30 minutes: the residue is NMT 10 mg (about 0.010%).

4-chlorobenzene sulfonamide $ClC_6H_4SO_2NH_2$ White to pale yellow, odorless, crystalline powder. Soluble in acetone.

Purity *Related substances*: Dissolve 0.60 g of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL. Perform the test with 5 μL of this solution as directed in the Purity (5) under Chlorpropamide; no spot other than the principal spot with the R_f value of about 0.5 appears.

***p*-Chlorobenzenesulfonamide** See 4-chlorobenzenesulfonamide.

2-Chlorobenzoic acid $C_7H_5ClO_2$ [First Class]

4-Chlorobenzoic acid ClC_6H_4COOH White crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically insoluble in water.

Melting point: 238 to 242 ℃
Content: NLT 99.0%.
Assay: Dissolve about 0.3 g of 4-chlorobenzoic acid, accurately weighed, n 30 mL of neutralized ethanol, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.657 mg of $C_7H_5ClO_2$

***p*-Chlorobenzoic acid** See 4-chlorobenzoic acid.

4-Chlorobenzophenone $C_{13}H_9ClO$ [Special Class]

1-Chlorobutane $CH_3(CH_2)3Cl$ Clear, colorless liquid. Miscible with ethanol (95) or ether, and practically insoluble in water.

Boiling point: About 78 ℃
Refractive index n_D^{20}: 1.041 to 1.045
Specific gravity d_{20}^{20} : 0.884 to 0.890

Chlorobutanol $Cl_3CC(CH_3)_2OH$ [See monograph, Part II]

Chloroform $CHCl_3$ [Special Class]

Chloroform for Karl Fischer titration See the Water Determination under the General Test.

Chloroform, ethanol-free Mix 20 mL of chloroform with 20 mL of water by gently shaking for 3 minutes, separate the chloroform layer, wash it with 20 mL each of water 2 times, filter with a dry filter paper, add 5 g of anhydrous sodium sulfate, shake well for 5 minutes for mixing, allow to stand for 2 hours, and then filter it with a dry filter paper. Prepare before use.

4-Chlorophenol ClC_6H_4OH White to slightly red crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol, chloroform, ether or glycerin, and sparingly soluble in water.

Melting point: About 43 ℃
Content: NLT 99.0%.

Assay: Add water to about 0.2 g of 4-chloropheno, accurately weighed, to make exactly 100 mL. Transfer 25 mL of this solution, accurately measured, to an iodine flask, add 20 mL of 0.1 mol/L bromine solution exactly and then 5 mL of hydrochloric acid to it. Immediately stopper the flask, mix for 30 minutes with occasional shaking, and then allow to stand for 15 minutes. Then, add 5 mL of potassium iodide solution (1 in 5), immediately stopper the flask, shake well for mixing, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L bromine VS
= 3.2140 mg of C_6H_5ClO

Packaging and storage: Preserve in light-resistant, tight containers.

***p*-Chlorophenol** See 4-chlorophenol.

(2-Chlorophenyl)-diphenylmethanol for thin layer chromatography See (2-chlorophenyl)-diphenylmethanol, for thin layer chromatography.

Chloroplatinic acid See hexachloroplatinic(IV) acid hexahydrate.

Chloroplatinic acid TS See hexachloroplatinic(IV) acid TS.

Chloroplatinic acid-potassium iodide TS See hexachloroplatinic(IV) acid-potassium iodide TS.

Chlorothiazide $C_7H_6ClN_3O_4S_2$ White crystalline powder. Odorless and tasteless. Freely soluble in *N*,*N*-dimethylformamide, slightly soluble in ethanol, acetone or pyridine, and very slightly soluble in water.

Method of preparation: Add 100 mL of diluted methanol (1 in 2) to 1 g of chlorothiazide and heat it to dissolve. Filter it while it is still warm, allow to stand overnight in a cold place, collect the precipitated crystals therefrom by filtering, and wash it with a small volume of diluted ethanol (1 in 3). Additionally, dry the collected crystals from recrystallization obtained by proceeding with the method and the original crystals at 105 ℃ for 2 hours.

Purity *Related substances*: Dissolve 60 mg of Chlorothiazide in methanol to make exactly 50 mL, and use this solution as the test solution. Perform the test with 10 μL of this solution as directed under the Liquid Chromatography according to the operating conditions in Assay of Hydrochlorothiazide: no peak other than that of Chlorothiazide appears. Adjust the detection sensitivity so that the peak height of chlorothiazide obtained from 10 μL of a 100 mL solution prepared by adding methanol to 2.0 mL of the test solution is about 1% of the full scale. Adjust the time span of measurement to be about 2 times the retention time of chlorothiazide after the solvent peak.

Chlorpheniramine maleate $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ [See monograph, Part I]

Chlorpropamide [See monograph, Part I on Chlorpropamide. When dried, it contains NLT 99.0% of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$).]

Cholesterol $C_{27}H_{45}OH$ [See monograph, Part II]

Cholesteryl *n*-heptylate $C_{34}H_{58}O_2$ [First Class]

Choline chloride $[(CH_3)_3NCH_2CH_2OH]Cl$ White crystalline powder.

Melting point: 303 to 305 ℃ (with decomposition)
Water: NMT 1 mg per g.

Chondroetinase ABC solution, pH 8.0 Dilute chondroitinase ABC enzyme with 50 mM tris buffer solution (pH 8.0) to make 0.001 unit/μL (sodium chondroitin sulfate, sodium chondroitin sulfate capsule).

Chromic acid-sulfuric acid TS Saturate sulfuric acid with chromium (VI) oxide.

Chromium trioxide CrO_3 [Chromium trioxide (Anhydrous chromic acid), Special class]

Chromium trioxide TS Dissolve 3 g of chromium trioxide in water to make 100 mL.

Chromium(VI) oxide CrO_3 Dare purple, thin acicular or columnar crystals or hard masses.

Identification: To 5 mL of an aqueous solution of chromium(VI) oxide (1 in 50), add 0.2 mL of lead acetate (II) TS; a yellow precipitate is produced and the precipitae does not dissolve on additon more of acetic acid.

Chromium(VI) oxide TS Dissolve 3 g of chromium(VI) oxide in water to make 100 mL.

Chromotropic acid See chromotropic acid disodium dihydrate.

Chromotropic acid disodium dihydrate $C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$ [Special Class] Store away from light..

Chromotropic acid TS Carefully add 68 mL of sulfuric acid to 30 mL of water, cool it, and add 100 mL of water. To this solution, add 50 mg of chromotropic acid disodium salt dihydrate to dissolve. Store away from light.

Chromotropic acid TS, concentrated Suspend 0.5 g of chromotropic acid disodium salt dihydrate in 50 mL of sulfuric acid, centrifuge it, and use the supernatant. Prepare before use.

Chymotrypsin solution Dissolve 0.1 g of chymotrypsin in 10 mL of water (chymotrypsin for injection).

Cinchonidine $C_{19}H_{22}N_2O$ White crystals or crystalline powder. Soluble in ethanol, methanol or chloroform, sparingly soluble in ether, and practically insoluble in water.
A solution of cinchonidine in ethanol (1 in 100) is levorotatory.

Melting point: About 207 ℃
Content: NLT 98.0%.

Assay: Weigh accurately about 0.3 g of cinchonidine, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate the resulting solution with 0.1 mol/L perchloric acid VS (indicator: 3 drops of methylrosaniline chloride TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.720 mg of $C_{19}H_{22}N_2O$

Cinchonine $C_{19}H_{22}N_2O$ White crystal or crystalline powder.

Identification: Dissolve 1 g of cinchonine in 20 mL of hydrochloric acid solution (1 in 4) and add 2 mL of potassium hexacyanoferrate(II) TS; a yellow precipitate is formed, which dissolves when heated and crystals precipitate when left to cool down.

Purity *Cinchonidine and quinine*: To 1 g of cinchonine, add 30 mL of water. Then, add hydrochloric acid solution (2 in 3) dropwise until dissolved and neutralize it with ammonia TS. Add 10 mL of sodium tartrate solution (1 in 2) to this solution, boil it, and allow it to stand for 1 hour: no precipitate is formed.

Content: NLT 98.0%.

Assay: Weigh accurately 0.3 g of cinchonine, dissolve in 50 mL of acetic acid (100), and titrate the resulting solution with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
14.72 mg of $C_{19}H_{22}N_2O$

Citrate buffer solution, pH 3.0 Dissolve 1.67 g of citric acid monohydrate and 1.47 g of sodium hydrogen phosphate docahydrate in water to make 100 mL.

Citrate buffer solution, pH 4.4 Add 88.2 mL of 0.2 mol/L disodium hydrogen phosphate TS to 111 mL of 0.1 mol/L citric acid solution.

Citrate buffer solution, pH 5.0 Dissolve 21 g of citric acid monohydrate in 1 mol/L sodium hydroxide TS, adjust the pH to 5.0, and add water to make 1 L.

Citrate buffer solution, pH 5.2 Mix 0.1 mol/L citric acid solution and 0.1 mol/L sodium citrate solution and adjust to pH 5.2.

Citrate buffer solution, pH 5.6 Mix 0.1 mol/L citric acid solution and 0.1 mol/L disodium hydrogen phosphate TS and adjust the pH to 5.6.

Citrate buffer solution, pH 6.2 Dissolve 15.3 g of citric acid monohydrate and 44.5 g of sodium hydrogen phosphate docahydrate in 150 mL of water, adjust the pH to 6.2 with 5 mol/L sodium hydroxide solution, and add water to make 250 mL.

Citrate buffer solution, pH 7.5 Add 0.2 mol/L disodium hydrogen phosphate TS to 7.5 mL of 0.1 mol/L citric acid solution to make 100 mL.

Citrate-phosphate buffer solution, pH 5.2 Dissolve 15.22 g of disodium hydrogen phosphate in 536 mL of water and add about 464 mL of 2.1% citric acid solution to adjust the pH to 5.25. To 985 mL of this solution, add 15 mL of 0.393% copper sulfate solution.

Citrate-phosphate buffer solution, pH 6.0 Dissolve 61.0 g of anhydrous disodium dihydrogen phosphate and 11.0 g of citric acid in 800 mL of water, adjust the pH to 6.0 with 6 mol/L hydrochloric acid, and add water to make 1000 mL.

Citrate-phosphate buffer solution, pH 7.5 Weigh 19.6 g of sodium monohydrogen phosphate, 1 g of citric acid, and 0.5 g of disodium edetate hydrate, and add water to make 1000 mL.

Citric acid See citric acid monohydrate.

Citric acid (100) $C_6H_8O_7$ [Special class].

Citric acid buffer solution Weigh 26.67 g of sodium citrate, 40.70 g of sodium chloride, and 6.10 g of citric acid monohydrate and add water to dissolve. Add 1.0 g of Brij 35 and dissolve in water to make 100 mL (sodium chondroitin sulfate).

Citric acid buffer solution, pH 3.0 Dissolve 21 g of citric acid monohydrate in water, add 200 mL of 1 mol/L sodium hydroxide TS, and add water to make 1 L. Add 597 mL of 0.1 mol/L hydrochloric acid TS to 403 mL of this solution (terbinafine hydrochloride).

Citric acid buffer solution, pH 5.0 Dissolve 20.256 g of citric acid and 7.840 g of sodium hydroxide in water to make 500 mL.

Citric acid monohydrate $C_6H_8O_7 \cdot H_2O$ [Special class or See monograph, Part I on Citric Acid].

1 mol/L Citric acid TS for buffer solution See citric acid TS, 1 mol/L, for buffer solution.

Citric acid TS, 0.01 mol/L Dissolve 2.1 g of citric acid monohydrate in water to make 1000 mL.

Citric acid TS, 0.2 mol/L Dissolve 4.2 g of citric acid monohydrate in water to make 100 mL (Povidone, Povidone ophthalmic solution).

Citric acid-acetate buffer solution Mix 70 mL of 10 mol/L sodium hydroxide solution, 70 g of citric acid hydrate, and 50 mL of acetic acid (31), and add water to make 1000 mL.

Citric acid-acetic acid TS See Citric acid-acetic acid TS.

Citric acid-acetic acid TS To 1 g of citric acid monohydrate, add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve by shaking.

Citric acid-acetic anhydride TS Add 50 mL of acetic anhydride to 1 g of citric acid monohydrate and warm the mixture to dissolve. Prepare before use.

Citric acid-peptone solution Weigh 1 g of peptone, 8.5 g of sodium chloride, and 20 mg of sodium citrate and dissolve in water to make 1 L (Lactobacillus acidophilus bacteria tyndalized, lyophilized product).

Citric acid-phosphate buffer solution (pH 5.2) See citrate-phosphate buffer solution, pH 5.2.

Citric acid-phosphate-acetonitrile TS Weigh accurately 2.1 g of citric acid monohydrate, 13.4 g of dibasic potassium phosphate, and 3.1 g of potassium dihydrogen phosphate, and dissolve in 1000 mL of a mixture of water and acetonitrile (3 : 1).

Clay acid Neutrally occuring hydrous aluminum silicate. Greyish white powder (particle size of about 74 μm).

Loss on drying: NMT 10% (1 g, 105 ℃, 4 hours).

Water adsorption capacity: NLT 2.5%. Weigh accurately about 10 g of clay acid in a weighing bottle, put the bottle with the cap open in a container with a humidity of 80% made by sulfuric acid with specific gravity of 1.19, allow to stand for 24 hours, weigh the mass, and determine the capacity of the sample.

Clindamycin B $C_{17}H_{31}ClN_2O_5S$ [Special Class]

Clotrimazole $C_{22}H_{17}OH$ [See monograph, Part I]

Cloxazolam $C_{17}H_{14}Cl_2N_2O_2$ [See monograph, Part I]

Cobalt acetate tetrahydrate $Co(CH_3COO)_2 \cdot 4H_2O$ [Special class]

Cobalt acetate TS Dissolve methanol in 100 mg of cobalt acetate tetrahydrate to make 100 mL.

Cobalt chloride See cobalt (II) chloride hexahydrate.

Cobalt chloride TS Dissolve 2 g of cobalt (II) chloride hexahydrate in 1 mL of hydrochloric acid and add water to dissolve and make 100 mL (0.08 mol/L).

Cobalt chloride-ethanol TS Dissolve 0.5 g of cobalt (II) chloride hexahydrate, previously dried at 105°C for 2 hours, in ethanol (99.5) to make 100 mL.

Cobalt-uranyl acetate TS Dissolve 4 g of uranyl acetate and 3 g of acetic acid in water to make 50 mL and heat this solution to dissolve. To this solution, before it is cooled, add a 50 mL solution made by dissolving 2 g of cobalt acetate and 3 g of acetic acid in water, cool to 20 ℃, and filter the solution two hours later. (Crystallized Glucosamine Sulfate Identity (4) Sodium)

Cobalt (II) chloride hexahydrate $CoCl_2 \cdot 6H_2O$ [Special Class]

Cobalt (II) nitrate See cobalt (II) nitrate hexahydrate.

Cobalt (II) nitrate hexahydrate $Co(NO_3)_2 \cdot 6H_2O$ [Special Class]

Colorimetric reagent for protein TS Dissolve 0.1 g of coomassie brilliant blue G250 in 50 mL of ethanol, then add 100 mL of 85% phosphoric acid, add 850 mL of water, shake to mix, and then filter. (sodium chondroitin sulfate).

Compactin $C_{23}H_{34}O_5$ [Special Class]

Concentrated chromotropic acid TS See chromotropic acid TS, concentrated.

Concentrated diazobenzenesulfonic acid TS See diazobenzenesulfonic acid TS, concentrated.

Concentrated lactose broth, 2x See lactose broth, 2x concentrated.

Concentrated lactose broth, 3x See lactose broth, 3x concentrated.

Concentrated potassium iodide TS See Potassium iodide TS, concentrated.

Control plastic for implantation See plastic, for implantation control.

Copper Cu [Special class]

Copper (standard reagent) Cu [Standard reagent for volumetric analysis]

Copper carbonate See Copper carbonate monohydrate

Copper carbonate monohydrate $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ Blue to bluish green powder. Insoluble in water. Soluble in dilute acid, foaming. Dissolves in ammonia TS to exhibit deep blue color.

Purity (1) Chloride: NMT 0.036%.

(2) Sulfate: NMT 0.120%

(3) Iron: To 5.0 g of copper carbonate monohydrate, add excess ammonia TS to dissolve and filter. Wash the residue with ammonia TS, add dilute hydrochloric acid to dissolve, add excess ammonia TS, and filter again. Wash the residue with ammonia TS, dry to a constant mass; the amount is NMT 10 mg.

Copper chloride TS Dissolve 0.12 g of copper chloride ($CuCl_2 \cdot 2H_2O$) in 250 mL of water and add pyridine to it to make 500 mL. (diethanolamine glucuronate-betaine glucuronate-nicotinamide ascorbate injection)

Copper ethylenediamine TS, 1 mol/L Add 100 g of 1 mol/L copper(II) hydroxide to a 1000-mL thick reagent bottle with a 500 mL graduation, and add water to make 500 mL. Attach a rubber stopper with a separatory funnel for liquid injection, a glass column for nitrogen introduction, and a glass column for gas discharge to the reagent bottle. Adjust the position below the nitrogen introduction tube to about 1.3 cm above the bottom of the reagent bottle. Let nitrogen flow through the nitrogen introduction tube at a reduced pressure of about 14 kPa, and use an appropriate regulator if necessary to foam quietly, allowing air inside the reagent bottle to be replaced with nitrogen for about 3 hours. Allow the nitrogen flowing inside the reagent bottle to flow in the same manner to make sure that it discharges through the gas discharge tube, and slowly add 160 mL of ethylenediamine TS through the separatory funnel for liquid injection while cooling with flowing water. Remove the separatory funnel for liquid injection and seal the hole of the rubber stopper with a glass rod. Flow additional nitrogen for about 10 minutes to pressurize while maintaining a pressure of about 14 kPa of nitrogen atmosphere. Shake the reagent bottle occasionally and allow it to stand for about 16 hours. If necessary, use a glass filter in vacuum, and then preserve it under a nitrogen atmosphere. The concentration of copper(II) ion obtained through this process is about 1.3 mol/L. Determine the concentrations X (mol/L) of ethylenediamine and Y (mol/L) of copper(II) ions in this solution as directed under the Assay, adjust the values to X = 1.96 to 2.04, Y = 0.98 to 1.02, and X/Y = 1.96 to 2.04 by adding water, copper(II) hydroxide, or ethylenediamine TS, quantify again in the same manner, and use this solution as the test solution.

Assay 1) *Ethylenediamine* Take exactly 1 mL (V_1) of the prepared solution, add 60 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (pH Measurement, endpoint pH is about 8.4).

X: Concentration (mol/L) of ethylenediamine in the prepared solution

a: Volume (mL) of 0.1 mol/L hydrochloric acid VS consumed

N_1: Concentration (mol/L) of hydrochloric acid

Assay 2) *Copper(II) ion* Pipet 2 mL (V_2) of the prepared solution, add 20 mL of water, 3 g of potassium iodide and 50 mL of 2 mol/L sulfuric acid, shake the mixture for 5 minutes, and then titrate free iodine with 0.1 mol/L sodium thiosulfate VS. The endpoint is reached when the blue color, obtained by adding 3

mL of starch TS and 10 mL of ammonium thiocyanate solution (2 in 10) when the color of the solution turns pale yellow near the endpoint, is decolorized.

Y: Concentration of copper(II) ion in the prepared solution (mol/L)
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed
N_2: Concentration (mol/L) of sodium thiosulfate solution

Copper sulfate See Copper(II) sulfate pentahydrate

Copper sulfate TS See copper(II) sulfate TS.

Copper sulfate-ammonia TS Dissolve 0.4 g of copper(II) sulfate pentahydrate in 50 mL of a mixture of ammonia TS and citric acid solution (1 in 5).

Copper sulfate-pyridine TS Dissolve 4 g of copper(II) sulfate pentahydrate in 90 mL of water, then add 30 mL of pyridine. Prepare before use.

Copper tartrate TS Dissolve 2.5 g of anhydrous sodium carbonate, 2.5 g of potassium sodium tartrate tetrahydrate, 2.0 g of sodium bicarbonate and 20.00 g of anhydrous sodium sulfate in water to make exactly 100 mL. To 25 mL of this solution, add 1 mL of 15 w/v% copper sulfate solution for mixing, before use.

Copper(II) acetate See Copper (II) acetate monohydrate

Copper(II) acetate monohydrate $Cu(CH_3COO)_2 \cdot H_2O$ Blue-green crystals or crystalline powder.

Identification (1) Dissolve 1 g of Copper (II) Acetate Monohydrate in 10 mL of diluted sulfuric acid (1 in 2), and heat; the odor of acetic acid is perceptible.

(2) Dissolve 0.1 g of Copper (II) Acetate Monohydrate in 20 mL of water, and add 3 mL of ammonia water (28); a dark blue color is developed.

Copper(II) acetate TS, strong See copper(II) acetate TS, strong.

Copper (II) acetate TS, strong Dissolve 13.3 g of copper (II) acetate monohydrate in a mixture of 195 mL of water and 5 mL of acetic acid (31).

Copper (II) chloride See copper(II) chloride dihydrate.

Copper (II) chloride dihyrate $CuCl_2 \cdot 2H_2O$ [Special Class]

Copper (II) chloride-acetone TS Dissolve 0.3 g of copper (II) chloride dihydrate in acetone to make 10 mL.

Copper(II) hydroxide $Cu(OH)_2$ Light blue powder. Practically insoluble in water.

Content: NLT 95.0% as $Cu(OH)_2$.

Assay: Weigh accurately about 0.6 g of copper(II) hydroxide, dissolve in 3 mL of hydrochloric acid and water to make exactly 500 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of ammonium chloride solution (3 in 5), 3 mL of diluted ammonia water (28) (1 in 10), and 0.05 g of murexide-sodium chloride indicator, and titrate the resulting solution with 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS. The endpoint of the titration is when the color of the solution changes from yellowish green to reddish purple.

Each mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 0.9756 mg of $Cu(OH)_2$

Copper (II) nitrate $Cu(NO_3)_2$ [Special Class]

Copper (II) nitrate solution Dissolve 1 g of copper (II) nitrate in water to make 100 mL.

Copper(II) sulfate See copper(II) sulfate pentahydrate

Copper(II) sulfate pentahydrate $CuSO_4 \cdot 5H_2O$ [Special class]

Copper(II) sulfate solution, alkaline Dissolve 150 g of potassium bicarbonate, 101.4 g of anhydrous potassium carbonate and 6.93 g of copper(II) sulfate pentahydrate in water to make 1000 mL.

Copper(II) sulfate TS Dissolve 12.5 g of copper(II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L).

Copper(II) sulfate, anhydrous $CuSO_4$ [Special class]

Corn oil [See monograph, Part II].

Cortisone acetate $C_{23}H_{30}O_6$ [See monograph, Part II on Cortisone Acetate]

Cotton wool [See monograph, Part II]

Cottonseed oil Prepared by purifing the non-volatile oils obtained from the seeds of *Gossypium hirsutum* Linné(*Gossypium*) or other congeneric plants. A pale yellow oily liquid, which is odorless. Miscible with ether, chloroform, hexane or carbon disulfide. Slightly soluble in ethanol (95).

Refractive index n_D^{20}: 1.472 to 1.474
Specific gravity d_{20}^{20}: 0.915 to 0.921
Saponification value: 190 to 198
Acid value: NMT 0.5
Iodine value : 103 ~ 116

Cresol $CH_3C_6H_4(OH)$ [See monograph, Part II]

Cresol red $C_{21}H_{18}O_5S$ [Special Class]

Cresol red TS Dissolve 100 mg of cresol red in 100 mL of ethanol (95), and if necessary, filter.

***m*-cresol** $CH_3C_6H_4(OH)$ [First Class]

Crystal violet See methylrosaniline chloride.

Crystal violet TS See methylrosaniline chloride TS.

Crystallized trypsin To trypsin obtained from of the bovine pancreas, add trichloroacetic acid to precipitate, and use ethanol (95) to recrystallize. White to yellowish white crystal or powder, having no odor. Freely soluble in water or sodium tetraborate-calcium chloride buffer solution, pH 8.0.

Content: NLT 45 FIP Units of trypsin per mg.

Cu-PAN To prepare Cu-PAN, mix 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) and 11.1 g of ethylenediaminetetraacetic acid

copper(II) disodium tetrahydrate. A gray-yellow, gray-reddish brown, or pale grayish purple powder.

Purity *Clarity and color of solution*: Dissolve exactly 50 mL of diluted dioxane (1 in 2) in 0.50 g of Cu-PAN; the resulting solution is yellowish brown and clear.

Absorbance: Weigh 0.5 g of Cu-PAN, dilute it, and dissolve in dioxane (1 in 2) to make exactly 50 mL. Pipet 1 mL of this solution, and add ethanol to make exactly 100 mL. Perform the test with this solution as directed under the Ultraviolet-visible Spectroscopy, using water as the blank; the absorbance at the wavelength of about 470 nm is NLT 0.48.

Cu-PAN TS Add 100 mL of diluted dioxane (1 in 2) to 1 g of Cu-PAN and dissolve.

Cupferron $C_6H_9N_3O_2$ [Cupferron (nitrosophenylhydroxylamine ammonium salt), Special Class]

Cupferron TS Dissolve 6 g of cupferron in water to make 100 mL. Prepare before use.

Cupric acetate See copper (II) acetate monohydrate.

Cupric acetate TS, stronger See copper(II) acetate TS, strong.

Cupric chloride See copper (II) chloride dihydrate.

Cupric chloride-acetone TS See copper (II) chloride-acetone TS.

Cupric hydroxide See copper(II) hydroxide.

Curcumin $C_{21}H_{20}O_6$ [Special Class]

Curcumin TS Dissolve 0.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare before use.

Cyanoacetic acid $C_3H_3NO_2$ White to pale yellow crystal. Very soluble in water.

Content: NLT 99.0%.

Assay: Weigh accurately about 300 mg of cyanoacetic acid, add 25 mL of water and 25 mL of ethanol to dissolve, and titrate the resulting solution with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 85.06 mg of $C_3H_3NO_2$

Cyanogen bromide TS See cyanogen bromide TS.

Cyanogen bromide TS Add 1 mL of bromine to 100 mL of water cooled with ice, shake vigorously to mix, and add potassium cyanide TS cooled with ice dropwise until the color of bromide is decolorized.

Cyanogen bromide TS for thiamine assay See cyanogen bromide TS, for thiamine assay.

Cyanogen bromide TS, for thiamine assay See cyanogen bromide TS, for thiamine assay.

Cyanogen bromide TS, for thiamine assay Add 2 mL of bromine in 100 mL of ice-cooled water, shake vigorously to mix, and add ice-cooled potassium thiocyanate TS dropwise until the color of bromine disappears. To this solution, add diluted8 mol/L sodium hydroxide TS (1 in 2), cooled with ice, to adjust the pH to 6, and add water to make 250 mL. Prepare this test solution in a fume hood before use. This test solution emits highly toxic fumes, so be careful not to inhale them when handling.

Cyanopropylphenyl dimethylpolysiloxane gum for gas chromatography Prepared for gas chromatography.

14% Cyanopropylphenyl-86% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

6% Cyanopropylphenyl- 94% Dimethylpolysiloxane gum for gas chromatography Prepared for gas chromatography.

Cyanopropylsilyl silica gel for liquid chromatography A silica get for liquid chromatography that is combined with cyanopropyl group.

Cyanuric chloride TS While cooling 60 mL of ethanol (95) in an ice bath, add 3.4 mL of acetic acid (100), 10 mL of sodium cyanide TS and 25 mL of toluenesulfonchloroamide sodium solution (7 in 50), shake for mixing, and then add water to make 100 mL. Use within 1 hours of preparation.

Cyclohexane C_6H_{12} [Special class].

3-Cyclohexylpropionic acid $C_9H_{16}O_2$ Clear liquid.
Refractive index n_D^{20}: About 1.4648
Boiling point: 100.3 ℃
Specific gravity d_{20}^{20}: About 0.998

Cycloserine reaction TS Mix equal volumes of sodium pentacyanonitrosylferrate(III) dihydrate solution (1 in 25) and sodium hydroxide solution (4 in 25). Preserve this solution in a brown bottle and use within 24 hours.

***L*-Cysteine hydrochloride monohydrate** $HSCH_2CH(NH_2)COOH \cdot H_2O$ [Special class].

***L*-Cysteine hydrochloride** See L-cysteine hydrochloride monohydrate.

***L*-Cystine** $HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$ [Special class].

DEAE-crosslinked dextran anion exchanger (Cl type), weakly basic A weakly basic anion in which a diethylamino ether group is introduced to the gel filtration carrier crosslinked dextran.

***N*-Demethyl erythromycin** $C_{36}H_{65}NO_{13}$ White to bright yellowish white powder.

***N*-Demethyl roxithromycin** $C_{40}H_{74}N_2O_{15}$ White powder.

Identification: Determine the infrared absorption spectrum as directed in the solution method under the Mid-infrared Spectroscopy, using fixed cells 0.1 mm in thickness made of potassium bromide with a solution of N-demethyl roxithromycin in chloroform (1 in 20) as the test solution; it exhibits absorption at the wave numbers of about 3600 cm^{-1}, 3520 cm^{-1}, 3450 cm^{-1}, 3340 cm^{-1}, 1730 cm^{-1}, and 1627 cm^{-1}.

Deoxyribonucleic acid TS Dissolve 0.15 g of sodium deoxycholate in 75 mL of mixed peptone TS on a water bath at 55 ℃ to 60 ℃ within 75 minutes. After cooling, add mixed peptone TS to make 100 mL, add 0.6 mL of magnesium sulfate TS to this mixture, and mix. Preserve at -12 ℃ to 8 ℃, and use within 6 weeks. However, when conducting the potency test for streptodornase, add standard streptodornase solution to the sample. Plot the reciprocal of the relative viscosity obtained on the ordinate, and plot the measurement time on the abscissa. Confirmed that a straight line is formed in advance (streptokinase, streptodornase).

2'-deoxyuridine for liquid chromatography See 2'-deoxyuridine, for liquid chromatography.

Desfibrinated rabbit blood Draw 100 mL of blood from a rabbit, collect in a flask, add about 20 glass sphere (8 mm in diameter), and shake carefully for 5 minutes to mix. Then, filter with absorbent gauze. Prepare before use.

Deuterated chloroform $CDCl_3$ Prepared for nuclear magnetic resonance spectroscopy

Deuterated chloroform for nuclear magnetic resonance spectroscopy $CDCl_3$ Prepared for nuclear magnetic resonance spectroscopy.

Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy $(CD_3)_2SO$ Prepared for nuclear magnetic resonance spectroscopy.

Deuterated formic acid DCOOD Deuterated formic acid with the purity of NLT 96.0%

Deuterated solvents for nuclear magnetic resonance spectroscopy Prepared for nuclear magnetic resonance spectroscopy. Deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide [$(CD_2)_2SO_4$], deuterium water(D_2O), deuterated pyridine (C_5H_5N), etc.

Deuterium oxide for nuclear magnetic resonance spectroscopy D_2O Prepared for nuclear magnetic resonance spectroscopy.

Devarda's alloy [Special class]

1,3-Di-(4-pyridyl)propane $C_{13}H_{14}N_2$ Pale yellow powder.

Melting point: 61 to 62℃

Water: NMT 0.1% (1 g).

2,6-Di-*t*-butylcresol TS Dissolve 0.1 g of 2,6-di-t-butylcresol in ethanol (95) to make 10 mL.

2,6-Di-*t*-butylcresol TS Dissolve 0.1 g of 2,6-di-t-butylcresol in ethanol (95) to make 10 mL.

2,6-Di-tert-butyl-*p*-cresol See 2,6-di-*t*-butylcresol.

2,6-Di-tert-butyl-*p*-cresol TS See 2,6-di-*t*-butylcresol TS.

Diacetyl $CH_3COCOCH_3$ Yellow to yellowish green clear liquid, having a strong, pungent odor. Miscible with ethanol or ether and freely soluble in water.

Refractive index n_D^{20}: 1.390 to 1.398

Boiling point: 85 ℃ to 91 ℃

Specific gravity d_{20}^{20}: 0.98 to 1.00

Congealing temperature: -2.0 ℃ to -5.5 ℃

Purity *Clarity and color of solution*: Dissolve 1.0 g of diacetyl in 10 mL of water; the resulting solution is clear.

Content: NLT 95.0%.

Assay: Weigh accurately about 0.4 g of diacetyl, add exactly 75 mL of hydroxylamine TS, and heat on a water bath for 1 hour under a reflux condenser. After cooling, titrate the excess hydroxylamine with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS). However, the endpoint of the titration is when this solution changes from blue to green and then finally to yellowish green. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.5 mol/L hydrochloric acid VS
= 21.523 mg of $C_4H_6O_2$

Diacetyl TS Dissolve 1 mL of diacetyl in water to make 100 mL, then take 5 mL of this solution, and add water to make 100 mL. Prepare before use.

Diamine chloride TS Take 60 mL of ethanol (95), add 3.4 mL of acetic acid (100), 10 mL of sodium cyanide TS and 25 mL of toluenesulfonchloroamide sodium solution (7 in 50) while cooling, shake for mixing, and then add water to make 100 mL. Use within 1 hour after preparation.

3,3'-Diaminobenzidine tetrahydrochloride $(NH_2)_2C_6H_3C_6H_3(NH_2)_2 \cdot 4HCl$ White needle-like crystal. Soluble in water. Stable in organic solvent at ordinary temperature but unstable in aqueous solution. Preserve the aqueous solution in a refrigerator.

Purity *Insoluble substances*: Dissolve 2 g of 3,3'-diaminobenzidine tetrahydrochloride in 100 mL of water, and immediately filter; the insoluble substance is NMT 1 mg (0.05%).

Residue on ignition: NMT 0.05% (2 g).

Selenium detection sensitivity: Dissolve 1.633 g of selenious acid (H_2SeO_3) in water, add water to make 1000 mL to obtain a solution containing 0.010 mg of selenium per mL. Add 2 mL of 3,3'-diaminobenzidine tetrahydrochloride solution (1 in 200), and allow to stand for 30 to 50 minutes. Adjust the pH to 6 to 7 with ammonia TS. Transfer this solution into a 125-mL separatory funnel, add 10 mL of toluene, and shake vigorously for 30 seconds to mix; the toluene layer exhibits a yellow color. Proceed with the diaminobenzidine tetrahydrochloride control solution which does not contain selenium RS in the same manner; the toluene layer has no color.

2,3-Diaminonaphthalene $C_{10}H_{10}N_2$ Pale yellowish brown crystal or powder. Slightly soluble in ethanol or ether and practically insoluble in water.

Melting point: 193 ℃ to 198 ℃

Sensitivity: Pipet 40 mL each of nitric acid (1 in 60) diluted as the selenium standard solution and the blank test solution, separately transfer into beakers, and add ammonia water (28) to adjust the pH to 1.8 to 2.2, respectively. Then, add 0.2 g of hydroxylamine hydrochloride, gently shake to dissolve, add 5 mL of 2,3-diaminonaphthalene TS, shake to mix, and then, allow to stand for 100 minutes. Transfer each solution to separatory funnels, wash the beakers with 10 mL of water, add washings to the separatory funnels, and then add 5.0 mL of cyclohexane. Shake well for 2 minutes, and extract. Take the cyclohexane layers, and centrifuge to remove moisture. Determine the absorbance of the cyclohexane solution obtained from the sele-

nium standard solution at a wavelength of 378 nm, using the cyclohexane solution obtained from the blank test solution as the blank; it is NLT 0.08.
Selenium standard solution Weigh accurately 40 mg of selenium (Se), add 100 mL of diluted nitric acid (1 in 2), dissolve by heating on a water bath, if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Prepare before use.
Each mL of this solution contains 0.04 μg of selenium (Se).

2,3-Diaminonaphthalene TS Dissolve 0.1 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylamine hydrochloride in 0.1 mol/L hydrochloric acid TS to make 100 mL.

2,4-Diaminophenol hydrochloride TS Dissolve 1 g of 2,4-diaminophenol hydrochloride and 20 g of sodium bisulfite in 100 mL of water, and filter, if necessary.

2,4-Diaminophenol hydrochloride $C_6H_8N_2O \cdot 2HCl$ Pale yellowish brown to gray, yellowish green crystalline powder. Freely soluble in water, slightly soluble in ethanol and practically insoluble in ether.

Purity *Clarity and color of solution*: Dissolve 1.0 g of 2,4-diaminophenol hydrochloride in 20 mL of water; the resulting solution is clear or slightly turbid.

Loss on drying: NMT 0.5% (1 g, 105 ℃, 3 hours)

Residue on ignition: NMT 0.5% (1 g).

Content: NLT 98.0%.

Assay: Weigh accurately about 0.2 g of 2,4-diaminophenol hydrochloride, dissolve in 50 mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 9.853 mg of $C_6H_8N_2O \cdot 2HCl$

Diammonium hydrogen citrate $C_6H_{14}N_2O_7$ [Special class].

***o*-Dianisidine** $C_{14}H_{16}N_2O_2$ White crystal. Soluble in ethanol, ether, and benzene, and insoluble in water.

Diatomaceous earth [First class]

Diatomaceous earth for chromatography White to pale gray in good quality.

Diatomaceous earth for gas chromatography Diatomaceous earth in good quality, prepared for gas chromatography.

Diatomaceous earth, for chromatography White to grayish white in good quality.

Diazo TS Weigh accurately 0.9 g of sulfanilic acid, add 0.9 mL of hydrochloric acid and 20 mL of water, and dissolve by heating. After cooling, filter, and add water to the filtrate to make exactly 100 mL. Pipet 1.5 mL of this solution, cool with ice, and then pipet 1 mL of sodium nitrite solution (1 in 20) and add dropwise while shaking to mix. Cool with ice for 10 minutes, and then add cold water to make exactly 50 mL. Preserve in a cold place, and use within 8 hours.

Diazo TS, for spraying Dissolve 2.5 g of sulfanilic acid in 900 mL of water, add 10 mL of hydrochloric acid, and shake to mix. Cool 10 mL of this solution with ice, add ice-cooled 10% sodium nitrite solution dropwise, shake to mix, and preserve in a refrigerator.

Diazobenzenesulfonic acid TS Weigh 0.9 g of sulfanilic acid, previously dried at 105 ℃ for 3 hours, dissolve in 10 mL of dilute hydrochloric acid by heating, and add water to make 100 mL. Take 3.0 mL of this solution, add 2.5 mL of sodium nitrite TS, and allow to stand for 5 minutes while cooling with ice. Then, add 5 mL of sodium nitrite TS and water to make 100 mL, and allow to stand in ice water for 15 minutes. Prepare before use.

Diazobenzenesulfonic acid TS, concentrated Weigh 0.2 g of sulfanilic acid, previously dried at 105 ℃ for 3 hours, dissolve in 20 mL of 1 mol/L hydrochloric acid TS by warming. Cool this solution with ice, and add 2.2 mL of a solution of sodium nitrite (1 in 25) dropwise while continuously stirring to mix. Allow to stand in ice water for 10 minutes, and then add 1 mL of amidosulfuric acid solution (1 in 20). Prepare before use.

Diazotized sulfanilic acid TS See diazobenzenesulfonic acid TS.

Dibasic ammonium phosphate $(NH_4)_2HPO_4$ [Special Class]

Dibasic potassium phosphate K_2HPO_4 [Special Class]

Dibasic potassium phosphate TS, 0.04 mol/L, pH 8.0 Dissolve 6.7 g of dibasic potassium phosphate in water to make 1000 mL and adjust the pH to 8.0 with phosphoric acid.

Dibasic potassium phosphate TS, 1 mol/L, for buffer solution Dissolve 174.18 g of dibasic potassium phosphate in water to make 1000 mL.

Dibasic potassium phosphate-citric acid buffer solution, pH 5.3 To 100 mL of 1 mol/L dipotassium hydrogen phosphate TS and 38 mL of 1 mol/L citric acid TS for buffer solution, add water to make 200 mL.

***N,N*-Dibenzylethylenediamine diacetate** White to slightly pale yellow crystal or crystalline powder.

Identification: Determine the infrared absorption spectrum of *N,N*-dibenzylethylenediamine diacetate as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wave numbers of about 1530 cm^{-1}, 1490 cm^{-1}, 1460 cm^{-1}, 1400 cm^{-1}, and 1290 cm^{-1}.

Content: NLT 99.0%.

Assay: Weigh accurately about 25 mg (potency) of *N,N*-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add a solution obtained by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5mL of this solution, add a mixture of a solution obtained by dissolving 1.02 g of anhydrous sodium dihydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1 : 1) to make exactly 20 mL, and use this solution as the test solution. Separately, weigh accurately about 8 mg of acetic anhydride, add 25 mL of methanol, and add a solution obtained by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of a solution obtained by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1 : 1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the test

solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area as directed under the automatic integration method, and calculate the amount of *N,N*-dibenzylethylenediamine diacetate as directed under the percentage peak area method.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column about 4.6 mm in internal diameter and about 250 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Constant temperature around 40 ℃

Mobile phase: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (11 : 7 : 2).

Flow rate: Adjust the flow rate so that the retention time of *N,N*-dibenzylethylenediamine diacetate is about 4 minutes.

System suitability

System performance: Weigh accurately an amount, equivalent to about 85,000 Units of benzathine penicillin G, dissolve in 25 mL of methanol, then add a solution obtained by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5mL of this solution, add a mixture of a solution obtained by dissolving 1.02 g of anhydrous disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1 : 1) to make exactly 20 mL. Proceed with 20 μL of this solution according to the above conditions; *N,N*-dibenzylethylenediamine diacetate and benzathine penicillin are eluted in this order with the resolution between these peaks being NLT 20.

System repeatability: Repeat the test 6 times with 20 μL each of the standard solution according to the above conditions; the relative standard deviation of the peak area of *N,N*-dibenzylethylenediamine diacetate is NMT 2.0%.

2,6-Dibromo-N-chloro-1,4-benzoquinonemonoimine $C_6H_2Br_2$: ClNO [Special class]

2,6-Dibromo-N-chloro-1,4-benzoquinone monoimine TS To 0.5 g of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine, add methanol to make 100 mL.

2,6-Dibromoquinonechlorimide See 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine.

2,6-Dibromoquinonechlorimide TS See 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS.

Dibucaine hydrochloride $C_{20}H_{29}N_3O_2 \cdot HCl$ [See monograph, Part I]

Dibutyl ether Clear and colorless liquid. Not miscible with water.

Specific gravity d_4^{20}: 0.768 to 0.771

1,2-Dichloroethane $ClCH_2CH_2Cl$ [Special class]

Dichlorofluorescein $C_{20}H_{10}Cl_2O_5$ Orange to reddish brown powder.

Identification (1) Dissolve 0.1 g of dichlorofluorescein in 10 mL of sodium hydroxide TS; the resulting solution exhibits an orange color. To this solution, add 10 mL of dilute hydrochloric acid to make acidic; a reddish orange precipitate forms.

(2) Dissolve 0.1 g of dichlorofluorescein in 10 mL of sodium hydroxide TS, and add 40 mL of water; the resulting solution exhibits a yellowish green fluorescence.

Dichlorofluorescein TS Dissolve 0.1 g of dichlorofluorescein in 60 mL of ethanol (95), add 2.5 mL of 0.1 mol/L sodium hydroxide solution, and add water to make 100 mL.

2,6-Dichloroindophenol sodium dihydrate $C_{12}H_6CI_2NNaO_2 \cdot 2H_2O$ [Special class]

2,6-Dichloroindophenol sodium TS To 0.1 g of 2,6-dichloroindophenol sodium dihydrate, add 100 mL of water, warm, and then filter. Use within 3 days.

2,6-Dichloroindophenol sodium TS for titration See 2,6-dichloroindophenol sodium TS, for titration.

2,6-Dichloroindophenol sodium TS, for titration See monograph on Ascorbic Acid, 2,6-dichloroindophenol sodium TS, for titration.

Dichloromethane CH_2Cl_2 [dichloromethane (methylene chloride), special class]

2,6-Dichlorophenolindophenol sodium See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenolindophenol sodium TS See 2,6-dichloroindophenol sodium TS.

2,6-Dichlorophenolindophenol sodium TS, for titration See 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichloroquinone chloroimide $C_6H_2Cl_3NO$ [Special class]

2,6-Dichloroquinone chloroimide See 2,6-dichloroquinone chloroimide.

2,6-Dichloroquinone chloroimide TS Dissolve 1 g of 2,6-dichloroquinone chloroimide in 100 mL of methanol (pyridoxine hydrochloride).

Dicyclohexyl phthalate $C_6H_4(COOC_6H_{11})_2$ A white, crystalline powder.

Melting point: 63 to 66 ℃

Purity *Clarity and color of solution*: Dissolve 1.0 g of dicyclohexyl phthalate in 20 mL of ethanol (95); the solution is clear and colorless.

***N,N*-Dicyclohexylcarbodiimide-anhydrous ethanol TS** See *N,N′*-Dicyclohexylcarbodiimide-ethanol (99.5) TS.

***N,N′*-Dicyclohexylcarbodiimide-ethanol (99.5) TS** Dissolve 6 g of *N,N′*-Dicyclohexylcarbodiimide in ethanol (99.5) to make 100 mL. Preserve in tight containers in a cold place.

***N,N′*-Dicyclohexylcarbodiimide** $C_6H_{11}N{=}C{=}NC_6H_{11}$ Colorless or white crystal or crystalline mass. Soluble in ethanol (95) but decomposes in water, producing a white precipitate.

Melting point: 35 ℃ to 36 ℃

Diethanolamine $C_4H_{11}NO_2$ Colorless and viscous liquid.

Melting point: 27 ℃ to 30 ℃

Water: NMT 0.1% (1 g).

Diethanolamine hydrochloride $C_4H_{11}NO_2 \cdot HCl$ Pale yellow liquid.

Refractive index n_D^{20}: 1.515 to 1.519
Specific gravity d_{20}^{20}: 1.259 to 1.263
Water: NMT 0.1% (1 g).

Diethyl ether See ether.

Diethyl ether, anhydrous See ether, anhydrous.

Diethyl terephthalate $C_6H_4(COOC_2H_5)_2$ White to pale brownish white crystals or masses.

***N,N*-Diethyl-*N*'-1-naphthylethylenediamine oxalate** $C_{18}H_{24}N_2O_4$ White crystalline powder.

Identification: Determine the infrared absorption spectrum of *N,N*-diethyl-*N*'-1-naphthylethylenediamine oxalate as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 3340 cm^{-1}, 2940 cm^{-1}, 1581 cm^{-1}, 1536 cm^{-1}, 1412 cm^{-1}, 789 cm^{-1}, 774 cm^{-1}, and 721 cm^{-1}.

Purity *Clarity and color of solution*: Dissolve 0.1 g of *N,N*-diethyl-*N*'-1-naphthylethylenediamine oxalate in 20 mL of water by warming; the resulting solution is clear.

***N,N*-Diethyl-*N*'-1-naphthylethylenediamine oxalate TS** Dissolve 1 g of *N,N*-diethyl-*N*'-1-naphthylethylenediamine oxalate in water to make 1000 mL.

***N,N*-Diethyl-*N*'-1-naphthylethylenediamine oxalate-acetone TS** Dissolve 1 g of *N,N*-diethyl-*N*'-1-naphthylethylenediamine oxalate in 100 mL of a mixture of acetone and water (1 : 1). Prepare before use.

Diethylamine $(C_2H_5)_2NH$ Clear, colorless liquid, having an amine-like characteristic odor. Miscible with water or ethanol. The aqueous solution is alkaline and readily absorbs carbon dioxide in the air.

Boiling point: 54 ℃ to 58 ℃, NLT 96 vol%.
Specific gravity d_{20}^{20}: 0.702 to 0.708
Content: NLT 99.0%.

Assay: Weigh accurately about 1.5 g of diethylamine in a flask containing exactly 30 mL of 0.5 mol/L sulfuric acid, and titrate excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank test in the same manner.

Each mL of 0.5 mol/L sulfuric acid VS
= 73.14 mg of $(C_2H_5)_2NH$

Silver *N,N*-diethyldithiocarbamate $C_5H_{10}AgNS_2$ [Special class]

Silver *N,N*-diethyldithiocarbamate $C_5H_{10}AgNS_2$ [Special class]

Diethylene glycol adipate ester for gas chromatography Prepared for gas chromatography.

Diethylene glycol dimethyl ether $(CH_3OCH_2CH_2)_2O$ Clear and colorless liquid. Miscible with water.

Boiling point: 158 ℃ to 160 ℃, NLT 95 vol%.
Specific gravity d_{20}^{20}: 0.940 to 0.950

Diethylene glycol monoethyl ether $C_2H_5(OCH_2CH_2)_2OH$ [2-(2-ethoxyethoxy)ethanol] Clear, colorless liquid, having about 203 ℃ of boiling point. Miscible with with water.

Refractive index n_D^{20}: 1.425 to 1.429
Specific gravity d_{20}^{20}: 0.990 to 0.995
Acid (as CH_3COOH): NMT 0.01%.

Diethylene glycol polyester succinate for gas chromatography Prepared for gas chromatography.

DiEthylene glycol succinate ester for gas chromatography Prepared for gas chromatography.

Difenidol hydrochloride $C_{21}H_{27}NO \cdot HCl$ [See monograph, Part I]

Digitonin $C_{56}H_{92}O_{29}$ White crystal or crystalline powder.

Optical rotation: -47 to -50 (2 g dried at 105 ℃ for 2 hours, 50 mL of diluted acetic acid (100) (3 in 4), 100 mm).

Sensitivity: Dissolve 0.5 g of digitonin in 20 mL of ethanol (95) by warming, and add ethanol (95) to make 50 mL. To 0.5 mL of this solution, add 10 mL of a solution of cholesterol in ethanol (95) (1 in 5000), cool to 10 ℃, and allow to stand for 30 minutes while occasionally vigorously shaking to mix; a precipitate forms.

1,3-Dihydroxynaphthalene $C_{10}H_6(OH)_2$ Purplish brown, crystals or powder. Freely soluble in water and ethanol.

Melting point: About 125 ℃

2,7-Dihydroxynaphthalene $C_{10}H_8N_2$ [Special class]

2,7-Dihydroxynaphthalene TS Dissolve 10 mg of 2,7-dihydroxynaphthalene in 100 mL of sulfuric acid.

Diisopropylamine $[(CH_3)_2CH]_2NH$ Colorless, clear liquid, having an amine-like characteristic odor. Miscible with water or ethanol. The solution in water is alkaline.

Specific gravity d_{20}^{20}: 0.715 to 0.722

Diltiazem hydrochloride $C_{22}H_{26}N_2O_4S \cdot HCl$ [See monograph, Part I]

Dilute acetic acid See acetic acid, dilute.

Dilute acetic acid See acetic acid, dilute

Dilute ammonia TS See ammonia TS.

Dilute ammonium iron(III) sulfate TS See ammonium iron(III) sulfate TS, dilute.

Dilute bismuth subnitrate-potassium iodide TS for spraying See bismuth subnitrate-potassium iodide TS, dilute, for spraying.

Dilute bromophenol blue TS See bromophenol blue TS, dilute.

Dilute *p*-dimethylaminobenzaldehyde-ferrous chloride TS See 4-dimethylaminobenzaldehyde-iron(III) chloride TS, dilute.

Dilute 4-dimethylaminobenzaldehyde-iron(III) chloride TS See 4-dimethylaminobenzaldehyde-iron(III) chloride, dilute.

Dilute ethanol See ethanol, dilute.

Dilute ferrous ammonium sulfate TS See ammonium iron(Ill) sulfate TS, dilute.

Dilute ferrous chloride TS See iron(Ill) chloride TS, dilute.

Dilute Folin TS (1 in 3) Take 1 mL of Folin TS, add 3 mL of water (newlase).

Dilute hydrochloric acid See hydrochloric acid, dilute.

Dilute hydrogen peroxide TS See hydrogen peroxide TS, dilute.

Dilute iodine TS See iodine TS, dilute.

Dilute iron-phenol TS See iron-phenol TS, dilute.

Dilute iron(III) chloride TS See iron(III) chloride TS, dilute.

Dilute lead subacetate TS See lead subacetate TS, dilute.

Dilute methyl red TS See methyl red TS, dilute.

Dilute nitric acid See nitric acid, dilute.

Dilute phenol red TS See phenol red TS, dilute.

Dilute potassium hydroxide-ethanol TS See potassium hydroxide-ethanol TS, dilute.

Dilute sodium hydroxide TS See sodium hydroxide TS, dilute.

Dilute sulfuric acid See sulfuric acid, dilute.

Dilute thymol blue TS See thymol blue TS, dilute.

Dilute vanadium pentoxide TS See vanadium(V) oxide TS, dilute.

Dilute vanadium(V) oxide TS See vanadium(V) oxide TS, dilute.

Diluted ethanol See ethanol, diluted

Dimedon $C_8H_{12}O_2$ White to pale yellow, crystalline powder.
Melting point: 145 ℃ to 149 ℃

Dimethyl phthalate $C_{16}H_{22}O_4$ Colorless, clear liquid, having a slight aroma.
Refractive index n_D^{20}: 1.491 to 1.493
Purity: To 6.0 mL of a solution of Dimethyl Phthalate in isooctane (1 in 100) add a solution of dimethyl phthalate in hexane (3 in 1000) to make 50 mL, and perform the test with 10 μL of this solution as directed under the Liquid Chromatography according to the conditions described in the Assay under Ergocalciferol or Cholecalciferol: any peak other than the major peak does not appear.

Dimethyl sulfoxide for absorption spectrum Colorless crystals or clear colorless liquid having a characteristic odor. Very hygroscopic.
Water: NMT 0.1%.
Congealing temperature: NLT 18.3 ℃.
Purity: Perform the test with Enzyme Diluent as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank. Measure the absorbance immediately after saturating with nitrogen; the wavelength is NMT 0.20 at 270 nm, NMT 0.09 at 275 nm, NMT 0.06 at 280 nm, and NMT 0.015 at 300 nm. Also, it does not show any special absorption at wavelengths of 260 to 350 nm.

Dimethyl yellow-oracet blue TS Dissolve 3 mg of dimethyl yellow and 3 mg of oracet blue in 100 mL of chloroform.

***N,N*-Dimethylacetamide** $CH_3CON(CH_3)_2$ Clear and colorless liquid.
Specific gravity: 0.938 to 0.945 (Method 3).
Melting point: 163 ℃ to 165 ℃ (with decomposition)
Water: NMT 0.2% (0.1 g, coulometric titration).
Purity: Perform the test with 3 µL of *N,N*-dimethylacetamide as directed under the Gas Chromatography according to the following conditions. Determine each peak area as directed under the automatic integration method, and calculate the amount of *N,N*-dimethylacetamide as directed under the percentage peak area method; it is NLT 98.0%.

Operating conditions
Detector: Flame ionization detector
Column: A fused silica column about 0.25 mm in internal diameter and about 30 m in length, coated with 0.5 µm in thickness with polyethylene glycol 20 M for gas chromatography.
Injection site temperature: Constant temperature around 70 ℃.
Detector temperature: Constant temperature around 200 ℃.
Column temperature: Maintain at 70 ℃ for 1 minute, then increase the temperature by 10 ℃ per minute until it reaches 200 ℃, and then maintain at 200 ℃ for 3 minutes.
Carrier gas: Helium
Flow rate: About 30 cm/sec.
System suitability
Test for required detectability: Weigh accurately 1.0 g of *N,N*-dimethylacetamide, and add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Adjust the peak area of *N,N*-dimethyactamide obtained from this solution to 40 to 60% of the full-scale.
System repeatability: Repeat the test 6 times with 3 μL each of the standard solution according to the above conditions; the relative standard deviation of the peak height of pyridine is NMT 2.0%.
Time span of measurement: About 3 times the retention time of *N,N*-dimethylacetamide.

***N,N*-Dimethylacetamide** $CH_3CON(CH_3)_2$ Clear and colorless liquid.
Specific gravity: 0.938 to 0.945 (Method 3).
Melting point: 163 ℃ to 165 ℃ (with decomposition)
Water: NMT 0.2% (0.1 g, coulometric titration).
Purity: Perform the test with 3 µL of *N,N*-dimethylacetamide as directed under the Gas Chromatography according to the following conditions. Determine each peak area as directed under the automatic integration method, and calculate the amount of *N,N*-dimethylacetamide as directed under the percentage peak area method; it is NLT 98.0%.

Operating conditions

Detector: Flame ionization detector

Column: A fused silica column about 0.25 mm in internal diameter and about 30 m in length, coated with 0.5 μm in thickness with polyethylene glycol 20 M for gas chromatography.

Injection site temperature: Constant temperature around 70 ℃.

Detector temperature: Constant temperature around 200 ℃.

Column temperature: Maintain at 70 ℃ for 1 minute, then increase the temperature by 10 ℃ per minute until it reaches 200 ℃, and then maintain at 200 ℃ for 3 minutes.

Carrier gas: Helium

Flow rate: About 30 cm/sec.

System suitability

Test for required detectability: Weigh accurately 1.0 g of *N*,*N*-dimethylacetamide, and add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Adjust the peak area of *N*,*N*-dimethyactamide obtained from this solution to 40 to 60% of the full-scale.

System repeatability: Repeat the test 6 times with 3 μL each of the standard solution according to the above conditions; the relative standard deviation of the peak height of pyridine is NMT 2.0%.

Time span of measurement: About 3 times the retention time of *N*,*N*-dimethylacetamide.

Dimethylamine $(CH_3)_2NH$ Colorless, clear liquid, having an amine-like characteristic odor. Miscible with water or ethanol (99.5). Alkaline.

Specific gravity d_{20}^{20}: 0.85 to 0.93

Content: 38.0 to 45.0%.

Assay: Weigh accurately about 1.0 g of dimethylamine, transfer into a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid, and titrate excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank test in the same manner.

Each mL of 0.5 mol/L sulfuric acid VS
= 45.08 mg of $(CH_3)_2NH$

Dimethylaminobenzaldehyde See 4-dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde $(CH_3)_2N_6H_4CHO$ [Special class]

***p*-Dimethylaminobenzaldehyde** See 4-dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde TS Dissolve 10 g of 4-dimethylaminobenzaldehyde in a cold mixture of 90 mL of sulfuric acid and 10 mL of water. Prepare before use.

4-Dimethylaminobenzaldehyde TS Dissolve 1.33 g of 4-dimethylaminobenzaldehyde in 50 mL of hydrochloric acid and 50 mL of methanol (crystallized glucosamine sulfate, crystallized glucosamine sulfate capsule).

4-Dimethylaminobenzaldehyde TS Dissolve 0.2 g of 4-dimethylaminobenzaldehyde in 0.5 mL of dilute hydrochloric acid and 20 mL of water, then add activated charcoal, vigorously shake, and filter (povidone).

4-Dimethylaminobenzaldehyde TS Dissolve 10 g of 4-dimethylaminobenzaldehyde in a cold mixture of 90 mL of sulfuric acid and 10 mL of water. Prepare before use (povidone ophthalmic solution).

4-Dimethylaminobenzaldehyde TS Dissolve 10 g of 4-dimethylaminobenzaldehyde in 100 mL of acetic acid (100) containing 12.5% of 10 mol/L hydrochloric acid. Add acetic acid (100) to dilute 10 times before use (sodium hyaluronate, sodium hyaluronate ocular injection).

***p*-Dimethylaminobenzaldehyde TS** See 4-dimethylaminobenzaldehyde TS.

***p*-Dimethylaminobenzaldehyde TS** See 4-dimethylaminobenzaldehyde TS.

4-Dimethylaminobenzaldehyde TS for spraying See 4-dimethylaminobenzaldehyde TS, for spraying.

***p*-Dimethylaminobenzaldehyde TS for spraying** See 4-dimethylaminobenzaldehyde TS for spraying.

4-Dimethylaminobenzaldehyde TS, for spraying Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20 mL of dilute sulfuric acid. Prepare before use.

***p*-Dimethylaminobenzaldehyde TS, for spraying** See 4-dimethylaminobenzaldehyde TS, for spraying.

***p*-Dimethylaminobenzaldehyde-ferrous chloride TS** See 4-dimethylaminobenzaldehyde-iron(III) chloride TS.

***p*-Dimethylaminobenzaldehyde-ferrous chloride TS, dilute** See 4-methylaminobenzaldehyde-Iron(III) chloride TS, dilute.

4-Dimethylaminobenzaldehyde-iron(III) chloride TS Dissolve 0.125 g of 4-dimethylaminobenzaldehyde in a cold mixture of 65 mL of sulfuric acid and 35 mL of water, and then add 0.05 mL of iron(III) chloride TS. Use within 7 days after preparation.

4-Dimethylaminobenzaldehyde-iron(III) chloride TS, dilute To 80 mL of water, add carefully 100 mL of 4-dimethylaminobenzaldehyde-iron(III) chloride TS and 0.15 mL of iron(III) chloride TS while cooling with ice.

4-Dimethylaminobenzylidenerhodanine $C_{12}H_{12}N_2OS_2$ [Special class]

***p*-Dimethylaminobenzylidenerhodanine** See 4-dimethylaminobenzylidenerhodanine.

4-Dimethylaminobenzylidenerhodanine TS Dissolve 20 mg of 4-dimethylaminobenzylidenerhodanine in acetone to make 100 mL.

***p*-Dimethylaminobenzylidenerhodanine TS** See 4-dimethylaminobenzylidenerhodanine TS.

4-Dimethylaminocinnamaldehyde $C_{11}H_{13}NO$ Orange crystal or crystalline powder, having a characteristic odor. Freely soluble in dilute hydrochloric acid, sparingly soluble in ethanol or ether and practically insoluble in water.

Melting point: 140 ℃ to 142 ℃.

Purity *Clarity and color of solution*: Dissolve 0.2 g of 4-Dimethylaminocinnamaldehyde in 20 mL of ethanol; the resulting solution is clear.

Loss on drying: NMT 0.5% (1 g, 105 ℃, 2 hours).

Residue on ignition: NMT 0.10% (1 g).

Nitrogen content: 7.8 to 8.1% (105 ℃, 2 hours, after drying, Nitrogen Determination).

***p*-Dimethylaminocinnamaldehyde** See 4-dimethylaminocinnamaldehyde TS.

4-Dimethylaminocinnamaldehyde TS To 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (1 in 2000), add 1 mL of acetic acid (100) before use.

***p*-Dimethylaminocinnamaldehyde TS** See 4-dimethylaminocinnamaldehyde TS.

Dimethylaminophenol meta isomer $C_8H_{11}NO$ Purple, black, gray, or brown crystalline solid.

Melting point: 83 ℃ to 85 ℃

Dimethylaminopropylsilyl silica gel for liquid chromatography Prepared for liquid chromatography.

Dimethylaniline $C_6H_5N(CH_3)_2$ [*N*,*N*-dimethylaniline, first class]

2,6-Dimethylaniline $C_8H_{11}N$ Colorless liquid. Sparingly soluble in water and soluble in ethanol.

Specific gravity d_{20}^{20}: 0.98

Dimethylformamide See *N*,*N*-dimethylformamide.

***N*,*N*-Dimethylformamide** $HCON(CH_3)_2$ [Special class]

Dimethylglyoxime $C_4H_8N_2O_2$ [Special class]

Dimethylglyoxime TS Dissolve 1 g of dimethylglyoxime in ethanol to make 100 mL.

Dimethylglyoxime TS Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL.

Dimethylglyoxime-thiosemicarbazide TS *Solution A*: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL. Prepare before use. *Solution B*: Dissolve 0. 1 g of thiosemicarbazide in 50 mL of water by warming, if necessary, and add diluted hydrochloric acid (1 in 2) to make 100 mL. Prepare before use. Mix 10 mL each of solution A and solution B, add diluted hydrochloric acid (1 in 2) to make 100 mL, allow to stand for 1 hour, and then use within 24 hours.

Dimethylmalonic acid $C_5H_8O_4$ Clear, colorless to pale yellow liquid.

Specific gravity d_{20}^{20}: 1.152 to 1.162

Water: NMT 0.3%.

Residue on ignition: NMT 0.1%.

Dimethylpolysiloxane gum for gas chromatography Prepared for gas chromatography.

Dimethylsulfoxide CH_3SOCH_3 [Special class]

1,3-Dinitrobenzene TS, alkaline Mix 1 mL of tetramethylammonium hydroxide TS and 140 mL of ethanol (99.5), take a portion of the mixture, titrate 0.01 mol/L hydrochloric acid VS, and then dilute the remaining solution with ethanol (99.5) to make a 0.008 mol/L solution. Mix 40 mL of this solution with 60 mL of a solution of 1,3-dinitrobenzene in benzene (1 in 20) before use.

***m*-Dinitrobenzene TS, alkaline** See 1,3-Dinitrobenzene TS, alkaline.

1,3-Dinitrobenzene $C_6H_4(NO_2)_2$ [Special class]

1,3-Dinitrobenzene TS Dissolve 1 g of 1,3-dinitrobenzene in 100 mL of ethanol (95). Prepare before use.

***m*-Dinitrobenzene TS** See 1,3-dinitrobenzene TS.

2,4-Dinitrochlorobenzene See 1-chloro-2,4-dinitrobenzene.

2,4-Dinitrofluorobenzene See 1-fluoro-2,4-dinitrobenzene.

2,4-Dinitrofluorobenzene TS See 1-fluoro-2,4-dinitrobenzene TS.

2,4-Dinitrophenol $C_6H_4N_2O_6$ Pale yellow crystal or crystalline powder.

Melting point: 110 ℃ to 114 ℃

2,4-Dinitrophenol TS Dissolve 0.5 g of 2,4-dinitrophenol in 100 mL of ethanol.

2,4-Dinitrophenylhydrazine saturated solution A saturated solution of 2,4-dinitrophenylhydrazine in low-carbonyl methanol solution. Use this solution within 1 week after preparation (nalbuphine hydrochloride).

2,4-Dinitrophenylhydrazine $(NO_2)_2C_6H_3NHNH_2$ [Special class]

2,4-Dinitrophenylhydrazine TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add water to make 100 mL. Filter, if necessary.

2,4-Dinitrophenylhydrazine TS Dissolve 0.1 g of 2,4-dinitrophenylhydrazine in 100 mL of 2 mol/L hydrochloric acid (allium sativum fluid extract).

2,4-Dinitrophenylhydrazine TS Dissolve 20 mg of 2,4-dinitrophenylhydrazine in a solution of trichloroacetic acid in benzene (1 in 20) to make 100 mL. Filter, if necessary. Prepare this test solution before use (methylephedrine hydrochloride).

2,4-Dinitrophenylhydrazine-benzene TS Dissolve 0.1 g of 2,4-dinitrophenylhydrazine in a solution of trichloroacetic acid in benzene (1 in 20) to make 100 mL. Filter, if necessary.

2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100 mL of diethylene glycol dimethyl ether by warming. After cooling, filter, if necessary.

2,4-Dinitrophenylhydrazine-ethanol TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add a mixture of aldehyde-free ethanol and water (1 : 3) to make 100 mL. Filter, if necessary.

2,4-Dinitrophenylhydrazine-hydrochloric acid TS Dissolve 100 mg of 2,4-dinitrophenylhydrazine in 2 mol/L hydrochloric

acid to make 100 mL (allantoin).

3,6-Dinitrophthalic acid compound with pyridine (1:1) solution Dissolve 1.5 g of 3,6-dinitrophthalic acid compound with pyridine (1:1) in 400 mL of water by warming, cool, and add water to make 500 mL. Preserve this solution in a brown bottle (taka-diastase N1).

Dinonyl phthalate $C_6H_4(COOC_9H_{19})_2$ Colorless to pale yellow, clear liquid.

Specific gravity d_{20}^{20}: 0.967 to 0.987

Acid value: NMT 2.

Dioxane See 1,4-dioxane.

1,4-Dioxane $C_4H_8O_2$ [diethylene dioxide, special class]

Diphenhydramine [See monograph, Part I]

Diphenyl $C_{12}H_{10}$ White crystal or crystalline powder, having a characteristic odor. Freely soluble in acetone or ether, soluble in ethanol and practically insoluble in water.

Melting point: 68 ℃ to 72℃

Purity: Dissolve 0.10 g of diphenyl in 5 mL of acetone, and use this solution as the test solution. Perform the test with 2 μL of this solution as directed under the Gas Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of diphenyl as directed under the percentage peak area method; it is NLT 98.0%.

Operating conditions

Detector: Flame ionization detector

Column: A glass column about 3 mm in internal diameter and about 2 m in length, packed with polyethylene glycol 20 M for gas chromatography coated with polyethylene glycol 20 M at the ratio of 10% (150 μm to 180 μm in particle diameter).

Column temperature: Constant temperature around 180 ℃

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of diphenyl is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diphenyl obtained from 2 μL of a solution, prepared by adding acetone to 1.0 mL of the test solution to make 100 mL, is 5 to 15% of the full scale.

Time span of measurement: About 3 times the retention time of diphenyl after the solvent peak.

Diphenyl phthalate $C_6H_4(COOC_6H_5)_2$ A white crystalline powder.

Melting point: 71 to 76 ℃

Purity *Related substances*: Dissolve 60 mg of diphenyl phthalate in 50 mL of chloroform, and use this solution as the test solution. Proceed with 10 μL of this solution as directed in the Assay under Tolnaftate Solution: any peak other than the major peak at the retention time of about 8 minutes and the peak of the solvent does not appear. Adjust the detection sensitivity so that the peak height of diphenyl phthalate obtained from 10 μL of the test solution is 50 to 100% of the full scale, and the time span of measurement is about twice the retention time of diphenyl phthalate after the solvent peak.

1,1-Diphenyl-2-picrylhydrazine $C_{18}H_{13}N_5O_6$ [Special class]

1,1-Diphenyl-2-picrylhydrazine solution Dissolve 8 mg of 1,1-diphenyl-2-picrylhydrazine in dimethylsulfoxide to make 50 mL (ciclopiroxolamine).

1,1-Diphenyl-4-piperidino-1-butene hydrochloride for thin layer chromatography See 1,1-diphenyl-4-piperidino-1-butene hydrochloride, for thin layer chromatography.

Diphenylamine $(C_6H_5)_2NH$ [Special class]

Diphenylamine TS Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

Diphenylamine TS Dissolve 7.5 g of diphenylamine in 300 mL of acetic acid (100), then add 180 mL of hydrochloric acid, mix well, and preserve in a brown bottle (dextran, hypromellose2910-dextran 70 ophthalmic solution).

Diphenylamine-acetic acid (100) TS Dissolve 1.5 g of diphenylamine in 1.5 mL of sulfuric acid and acetic acid (100) to make 100 mL.

Diphenylamine-acetic acid TS See diphenylamine-acetic acid (100) TS.

9,10-Diphenylanthracene $C_{26}H_{18}$ Yellow crystalline powder. Soluble in ether, and practically insoluble in water.

Melting point: About 248 ℃

Diphenylcarbazide See 1,5-diphenylcarbohydrazide.

Diphenylcarbazide TS See 1,5-diphenylcarbohydrazide TS.

Diphenylcarbazone $C_{13}H_{12}N_4O$ Yellowish red crystalline powder.

Identification: Determine the infrared spectrum as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1708 cm^{-1}, 1602 cm^{-1}, 1497 cm^{-1}, 1124 cm^{-1}, 986 cm^{-1}, 748 cm^{-1} and 692 cm^{-1}.

Packaging and storage: Preserve in light-resistant, tight containers.

Diphenylcarbazone TS Dissolve 1 g of diphenylcarbazone in ethanol to make 1000 mL.

1,5-diphenylcarbohydrazide $C_{13}H_{14}N_4O$ [Special class].

1,5-diphenylcarbohydrazide TS Dissolve 0.2 g of 1,5-diphenylcarbohydrazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9 : 1).

Diphenylether $C_{12}H_{10}O$ Colorless crystals, having a geranium-like aroma. Soluble in ethanol or ether, and practically insoluble in water.

Specific gravity d_{20}^{20}: 1.072 to 1.075

Boiling point: 254℃ to 259 ℃

Melting point: About 28 ℃

1 mol/L Dipotassium monohydrogen phosphate TS for buffer solution See dipotassium hydrogen phosphate TS, 1 mol/L, for buffer solution.

2,2´-Dipyridyl $C_{10}H_8N_2$ [Special class]

α,α'-Dipyridyl See 2,2'-dipyridyl.

Dische reagent Dissolve 1 g of diphenylamine in a mixture of 100 mL of acetic acid and 2.75 mL of sulfuric acid (deoxyribonuclease).

Disodium dihydrogen phosphate for pH measurement See disodium dihydrogen phosphate, for pH measurement.

Disodium hydrogen phosphate See disodium hydrogen phosphate dodecahydrate.

Disodium hydrogen phosphate-citric acid buffer solution, 0.05 mol/L, pH 6.0 To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS, add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL until the pH reaches 6.0

Disodium hydrogen phosphate dodecahydrate $Na_2HPO_4 \cdot 12H_2O$ [Special Class]

Disodium hydrogen phosphate, anhydrous Na_2HPO_4 [Special Class]

Disodium hydrogen phosphate, for pH measurement Na_2HPO_4 [Special Class]

Disodium hydrogen phosphate TS See disodium hydrogen phosphate TS, 0.3 mol/L.

Disodium hydrogen phosphate TS, 0.05 mol/L Dissolve 7.098 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Disodium hydrogen phosphate TS, 0.2 mol/L Dissolve 28.3928 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Disodium hydrogen phosphate TS, 0.3 mol/L Dissolve 12 g of disodium hydrogen phosphate dodecahydrate in water to make 100 mL.

Disodium hydrogen phosphate TS, 0.5 mol/L Dissolve 70.982 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 Dissolve 21.02 g of citric acid monohydrate in water to make 1000 mL. To this solution, add a solution prepared by dissolving 35.82 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL until the pH reaches 4.5.

Disodium hydrogen phosphate-citric acid buffer solution, pH 5.4 Dissolve 1.05 g of citric acid monohydrate and 2.92 g of disodium hydrogen phosphate dodecahydrate in 200 mL of water. If necessary, adjust the pH to 5.4 with phosphoric acid or sodium hydroxide TS.

Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 Dissolve 28.4 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. To this solution, add a solution prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL until the pH reaches 6.0 (volume ratio: about 63 : 37).

Disodium hydrogen phosphate-citric acid buffer solution, pH 7.5 To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS, add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL until the pH reaches 7.5.

Dissolved acetylene C_2H_2 [Special Class]

Distilled water for ammonium test See purified water, for ammonium test.

Distilled water for injection See distilled water, for injection.

Distilled water, for injection [See monograph, Part II]

1,1′-[3,3′-Dithiobis(2-methyl-1-oxopropyl)]-L-diproline $C_{18}H_{28}N_2O_6S_2$ White crystal or crystalline powder. Sparingly soluble in methanol, and practically insoluble in water.

Identification: Determine the infrared absorption spectrum of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wave numbers of about 2960 cm^{-1}, 1750 cm^{-1}, 1720 cm^{-1}, 1600 cm^{-1}, 1480 cm^{-1}, 1450 cm^{-1}, and 1185 cm^{-1}.

Purity *Related substances*: Dissolve 0.10 g of 1,1'-[3,3'-Dithiobis(2-Methyl-1-Oxopropyl)]-L-Diproline in exactly 10 mL of methanol, and use this solution as the test solution. Perform the test with the test solution as directed under the Thin Layer Chromatography. Spot 10 μL of the test solution on the thin plate made of silica gel for thin-layer chromatography. Then, develop the plate with a mixture of toluene and acetic acid (100) (13 : 7) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand for 30 minutes in a chamber filled with iodine vapors; no spots other than the principal spot with the R_f value of about 0.6 appear.

Content: NLT 99.0%.

Assay: Weigh accurately about 0.3 g of 1,1'-[3,3'-Dithiobis(2-Methyl-1-Oxopropyl)]-L-diproline, dissolve in 20 mL of methanol, add 50 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). However, the endpoint of the titration is when this solution changes from yellow to bluish green and then finally to blue. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.628 mg of $C_{18}H_{28}N_2O_6S_2$

5,5'-Dithiobis(2-nitrobenzoic acid) $C_{14}H_8N_2O_8S_2$ Yellow powder. Sparingly soluble in ethanol.

Melting point: About 242 ℃

Dithiothreitol $C_4H_{10}O_2S_2$ Crystal.

Melting point: About 42 ℃

Dithizone $C_6H_5NHNHCSN{:}NC_6H_5$ [dithizone (diphenylcarbazone), special class]

Dithizone solution, for extraction Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of ethanol (95). Preserve dithizone in a cold place. Before use, take the required amount of this solution, add half of its volume of diluted nitric acid (1 in 100), shake to mix, and use after discarding the aqueous layer.

Dithizone solution, for extraction See dithizone solution, for extraction.

Dithizone TS Dissolve 25 mg of dithizone in ethanol (95) to make 100 mL. Prepare before use.

Dragendorff TS for spraying See Dragendorff's TS, for spray.

Dragendorff-Munier stain TS Dissolve 176 g of bismuth acetate basic and 200 g of tartaric acid in 800 mL of water (A). Separately, dissolve 160 g of potassium iodide in 400 mL of water (B). Mmix 50 mL of a mixture of solution A and solution B (1 : 1), 100 mg of tartaric acid and 500 mL of water before use. Sodium dodecyl sulfate can be preserved for 3 weeks (oxapium iodide).

Dragendorff's TS To 0.85 g of bismuth subnitrate, add 10 mL of acetic acid (100) and 40 mL of water, shake vigorously, and use this solution as solution A. Dissolve 8 g of potassium iodide in 20 mL of water and use this solution as solution B. Immediately before use, mix equal volume of solution A, solution B, and acetic acid (100).
Preserve the solution A and solution B, protected from light.

Dragendorff's TS for spraying See Dragendorff's TS, for spraying.

Dragendorff's TS, for spraying To 4 mL of a mixture of Dragendorff's TS in equal volume of solution A and solution B, add 20 mL of diluted acetic acid (31) (1 in 5). Prepare before use.

Dragendorff-Munier stain TS Dissolve 176 g of bismuth acetate basic and 200 g of tartaric acid in 800 mL of water (A). Separately, dissolve 160 g of potassium iodide in 400 mL of water (B). Mmix 50 mL of a mixture of solution A and solution B (1 : 1), 100 mg of tartaric acid and 500 mL of water before use. Sodium dodecyl sulfate can be preserved for 3 weeks (oxapium iodide).

DT TS Mix 10 mL of thiosemicabazide TS and 10 mL of dimethylglyoxime TS, add dilute hydrochloric acid (1 in 2) to make 100 mL, and allow to stand for 1 hour before use.

DTNB TS Dissolve 100 mg of 5,5'-dithio-bis-2-nitrobenzoic acid in 25 mL of methanol (tiopronin).

Ehrlich TS Dissolve 1 g of dimethylaminobenzaldehyde in 100 mL of ethanol (99.5) and add 1.5 mL of hydrochloric acid.

Eikonogene TS Mix 0.5 g of 1-amino-2-naphthol-4-sulfonic acid with 195 mL of 15% sodium bisulfite solution, and dissolve it by adding 20% sodium sulfite solution dropwise. This TS is stable for 14 days (fructose-diphosphate magnesium hydrate).

EMB planar medium Melt eosin methylene blue agar medium by heating, and cool to about 50°C. Transfer about 20 mL of EMB planar medium to a Petri dish, and solidify horizontally. Place the dish with the cover slightly opened in the incubator to evaporate the inner vapor and water on the plate.

Endo medium Dissolve 1000 mL of normal agar medium on a water bath by warming to adjust the pH to 7.5 to 7.8, add 10 g of lactose monohydrate previously dissolved in a small volume of water, mix well, add 1 mL of fuchsin-ethanol TS, cool it to about 50 ℃, and then add freshly prepared sodium sulfite heptahydrate solution (1 in 10) dropwise until the color of the solution turns to pale red. The consumed amount of the sodium sulfite heptahydrate solution (1 in 10) is about 10 mL to 15 mL. Dispense this solution and tyndallize once daily on three successive days for 15 minutes at 100 ℃.

Endo plate medium Dissolve endo medium by heating, cool to about 50 ℃, take about 20 mL of it to a Petri dish, and solidify it horizontally. Place the dish with the cover slightly opened in an incubator to evaporate the inner steam or water on the plate.

Enterokinase solution Dissolve 500 g of trichloroacetic acid in 1 L of water (pancrease).

Enterokinase solution In a cold mortar, weigh accurately 25 mg of enterokinase (FIP-controlled, 1 mg = 1 FIP unit) (storage: 15 ℃), and mix it with 3 mL of 0.02 mol/L calcium chloride solution. To the above solution, add cold 0.02 mol/L calcium chloride solution to make 25 mL (1 mL = enterokinase 1 FIP unit) (storage: 0 ℃ ice water) (use within 1 hour after preparation) (pancreatinII).

Enzyme Diluent Dissolve 5.3 g of L-cysteinehydrochloride and 22 g of ethylenediaminetetraacetic acid disodium dihydrate in 900 mL of water, adjust the pH to 4.5 with 1 mol/L sodium hydroxide solution, and add water to make exactly 1 L. Prepare before use (bromelain).

Enzyme TS The supernatant is obtained as follows: Obtain an enzyme preparation from *Aspergillus oryzae* that is potent in starch saccharifying activity and power to digest phosphate ester. To 0.3 g of the enzyme preparation, add 10 mL of water and 0.5 mL of 0.1 mol/L hydrochloric acid TS, mix vigorously for a few minutes, and centrifuge. Prepare before use.

Eosin $C_{20}H_6Br_4Na_2O_5$ [First Class]

Eosin methylene blue agar medium To 10 g of casein peptone, 2 g of dibasic potassium phosphate and 25 g to 30 g of agar, add about 900 mL of water, and dissolve by boiling. To this solution, add 10 g of lactose, 20 mL of eosin solution (1 in 50, 13 mL of methylene blue solution (1 in 200), and warm water to make 1000 mL, mix it well, dispense the mixture, and sterilize it in an autoclave at 121 ℃ for NLT 20 minutes. Quickly immerse it in cold water for cooling. Alternatively, tyndallize once daily on three successive days for 15 to 30 minutes at 100 ℃.

Eosin Y See eosin.

Ephedrine hydrochloride $C_{10}H_{15}NO \cdot HCl$ [See monograph, Part I]

4-epi-oxytetracycline $C_{22}H_{24}N_2O_9$ Greenish brown to brown powder.

Purity Related substances: Dissolve 20 mg of 4-epi-oxytetracycline in 25 mL of 0.01 mol/L hydrochloric acid TS and use this solution as the test solution. Determine each peak area with 20 μL of this solution as directed in the Purity (1) under Oxytetracycline Hydrochloride by the automatic integration method and calculate according to the percentage peak area method : the sum of peak areas other than 4-epi-oxytetracycline is NMT 10%.

Eriochrome black T $C_{20}H_{12}N_3NaO_7S$ [Special Class]

Eriochrome black T TS Dissolve 0.3 g of eriochrome black T and 2 g of hydroxylamine hydrochloride in methanol to make 50 mL. Use within 1 week after preparation. Store away from light.

Eriochrome black T-sodium chloride indicator Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and grind the mixture until it becomes homogeneous powder.

Erythritol $C_4H_{10}O_4$ [Special Class]

Erythrocyte suspension, Type A Prepare a suspension containing 1 vol% of erythrocyte separated from human A-type blood in isotonic sodium chloride injection.

Erythrocyte suspension, Type B Prepare a suspension containing 1 vol% of erythrocyte separated from human B-type blood in isotonic sodium chloride injection.

Erythromycin B $C_{37}H_{67}NO_{12}$ White to bright yellow powder.

Purity *Related substances*: Dissolve 10 mg of Erythromycin B in 1 mL of methanol, add the mixture of pH 7.0 phosphate buffer solution and methanol (15 : 1) to make 5 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add the mixture of phosphate buffer solution and methanol (15 : 1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under the Liquid Chromatography according to the operating conditions in the Purity (4) Related substances under Erythromycin. Determine the peak areas of each solution by the automatic integration method: the sum of peak areas other than the peak area of erythromycin B in the test solution is not greater than the peak area of erythromycin B in the standard solution.

Erythromycin C $C_{36}H_{65}NO_{13}$ White to bright yellow powder.

Purity *Related substances*: Dissolve 10 mg of Erythromycin B in 1 mL of methanol, add the mixture of pH 7.0 phosphate buffer solution and methanol (15 : 1) to make 5 mL, and use this solution as the test solution. Pipet 1 mL of this solution, add a mixture of pH 7.0 phosphate buffer solution and methanol (15 : 1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the test solution and standard solution as directed under the Liquid Chromatography according to the operating conditions in 4) Related substances of the Purity under Erythromycin. Determine the peak areas of each solution by the automatic integration method: the sum of peak areas other than the peak area of erythromycin C in the test solution is not greater than the peak area of erythromycin C in the standard solution.

Ethanol See ethanol (95).

Ethanol (95) C_2H_5OH [Special Class]

Ethanol (99.5) C_2H_5OH [Special Class]

Ethanol for disinfection See ethanol, for disinfection.

Ethanol-free chloroform See chloroform, ethanol-free.

Ethanol-phosphate buffer solution Dissolve 0.29 g of sodium monohydrogen phosphate in 450 mL of water, add 550 mL of ethanol (95) and mix.

Ethanol, aldehyde-free Transfer 1000 mL of ethanol (95) to a glass-stoppered bottle, add a solution prepared by dissolving 2.5 g of lead(II) acetate trihydrate in 5 mL of water, and mix thoroughly. Separately, dissolve 5 g of potassium hydroxide in 25 mL of warm ethanol (95), cool, and add this solution gently, without stirring, to the first solution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the clear supernatant, and distill.

Ethanol, anhydrous See ethanol (99.5).

Ethanol, dilute To 1 volume of ethanol (95), add 1 volume of water. It contains 47.45 vol% to 50.00 vol% of C_2H_5OH.

Ethanol, diluted Prepare by using ethanol (99.5).

Note: It is desirable to use the expression: diluted ethanol (99.5).

Ethanol, for disinfection 〔See monograph, Part II〕

Ethanol, neutralized To a suitable quantity of ethanol (95), add 2 to 3 drops of phenolphthalein TS, then add 0.01 mol/L or 0.1 mol/L sodium hydroxide solution until a pale red color develops. Prepare before use.

Ethanol, saline injection solution To 1 volume of ethanol (95), add 19 volumes of isotonic sodium chloride injection.

Ethenzamide $C_9H_{11}NO$ [See monograph, Part I]

Ether $C_2H_5OC_2H_5$ [Special Class]

Ether, anhydrous $C_2H_5OC_2H_5$ [Special Class but with water NMT 0.01%.]

Ether, for anesthesia $C_2H_5OC_2H_5$ [See monograph, Part I]

Ethinylestradiol $C_2H_{24}N_2$ [See monograph, Part I]

3-Ethoxy-4-hydroxybenzaldehyde $C_9H_{10}O_3$ White to pale yellow crystalline powder. Freely soluble in ethanol (95) and slightly soluble in water.

Melting point: 76 ℃ to 78 ℃

Content: NLT 98.0%

Assay: Dry 3-Ethoxy-4-hydroxybenzaldehyde in a desiccator using phosphorus pentoxide as a desiccant for 4 hours, weigh accurately about 0.3 g of it, add *N*,*N*-dimethylformamide to dissolve, and titrate with 0.1 mol/L sodium methoxide solution (indicator: thymol blue TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide
= 16.62 mg of $C_9H_{10}O_3$

4-Ethoxyphenol $C_8H_{10}O_2$ White to pale yellowish brown crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

Melting point: 62 ℃ to 68 ℃

Purity: Dissolve 0.5 g of 4-Ethoxyphenol in 5 mL of ethanol (95), and use this solution as the test solution. Perform the test with 1 μL of this solution as directed under the Gas Chromatography according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of substance other than 4-ethoxyphenol by the percentage peak area method: it is NMT 2.0%.

Operating conditions

Detector: Thermal conductivity detector

Column: A column about 3 mm in internal diameter and about 2 m in length, packed with 180 μm to 250 μm diatomaceous earth for gas chromatography that is coated with methyl silicone polymer for gas chromatography in the ratio of 10%.

Column temperature: Constant temperature around 150°C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 4-ethoxyphenol is about 5 minutes.

Detection sensitivity: Adjust the sensitivity so that the peak height of 4-ethoxyphenol obtained from 1 μL of the test solution is NLT 50% of the full scale.

Time span of area measurement: About 3 times the retention time of 4-ethoxyphenol after the solvent peak.

***p*-Ethoxyphenol** See 4-ethoxyphenol.

Ethyl acetate $CH_3COOC_2H_5$ [Acetate]

Ethyl acetate See ethyl acetate.

Ethyl acetate See ethyl acetate.

Ethyl alcohol (95) See ethanol (95).

Ethyl aminobenzoate $C_9H_{11}NO_2$ [See monograph, Part I]

***η*-Ethyl caprylate** $C_{10}H_{20}O_2$ Clear and colorless liquid.

Specific gravity d_{20}^{20}: 0.864 to 0.871

Purity *Related substances*: Dissolve 0.1 g of η-Ethyl caprylate in 10 mL of dichloromethane and use this solution as the test solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 μL each of the test solution and standard solution (1) as directed under the Gas Chromatography according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the sum of peak areas other than η-ethyl caprylate from the test solution is not greater than the peak area of η-ethyl caprylate from the standard solution (1).

Operating conditions

For operating conditions other than the detection sensitivity and time span of measurement, proceed with the operating conditions in the Assay under Mentha Oil.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Make adjustments so that the peak area of η-ethyl caprylate obtained from 5 μL of the standard solution (2) can be determined by the automatic integration method. Also, adjust the peak height of η-ethyl caprylate obtained from 5 μL of the standard solution (1) 5 so that it is about 20% of the full scale.

Time span of measurement: 3 times the retention time of η-ethyl caprylate beginning after the solvent peak.

Ethyl carbamate $H_2NCOOC_2H_5$ [Ethyl carbamate (urethane), Special Class]

Ethyl cyanoacetate $NCCH_2COOC_2H_5$ Colorless or pale yellow, clear liquid with an aromatic odor.

Specific gravity d_{20}^{20}: About 1.08

Identification: To 0.5 mL of an ethyl cyanoacetate solution in ethanol (99.5) (1 in 10000), add 1 mL of a quinhydrone solution in dilute ethanol (99.5) (1 in 2) (1 in 20,000) and ammonia water (28) dropwise; the solution exhibits a bright blue color.

Ethyl formate $C_3H_6O_2$ [Special class]

Ethyl iodide C_2H_5I [Special Class]

Ethyl Paraoxybenzoate $HOC_6H_4COOC_2H_5$ [See monograph, Part II]

Ethyl propionate $CH_3CH_2COOC_2H_5$ A clear, colorless liquid.

Specific gravity d_4^{20}: 0.890 to 0.892

2-Ethyl-2-methylsuccinic acid $C_7H_{12}O_4$ White crystals.

Melting point: 101 ℃ to 103 ℃

Content: NLT 98.5%

Assay: Take exactly 80 mg of 2-Ethyl-2-methylsuccinic acid, add 30 mL of methanol to dissolve, and slowly add 40 mL of purified water. If the solution is turbid, only use 50 mL of methanol for dissolution. Perform assay using 0.1 mol/L sodium hydroxide TS according to potentiometric titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 16.02 mg of $C_6H_6O_4$

2-Ethyl-2-phenylmalondiamide $C_{11}H_{14}O_2N_2$ White, odorless crystals. Sparingly soluble in ethanol (95) and very slightly soluble in water.

Melting point: 120 ℃ (with decomposition)

Purity *Related substances*: Add pyridine 4 mL to 5.0 mg of 2-Ethyl-2-phenylmalondiamide, add 1 mL of bistrimethylsilylacetamide, shake well for mixing, and then heat at 100 ℃ for 5 minutes. Cool it, add pyridine to make exactly 10 mL, and use this solution as the test solution. Perform the test with 2 μL of 2-Ethyl-2-phenylmalondiamide as directed under the Gas Chromatography according to the operating conditions in Purity (3) under Primidone: no peak other than that of 2-Ethyl-2-phenylmalondiamid and solvent appears. The detection sensitivity is adjusted so that the peak height of 2-ethyl-2-phenylmalondiamide obtained from 2 μL of the test solution is about 80% of the full scale. The time span of peak measurement is about 2 times the retention time of 2-ethyl-2-phenylmalondiamide at the solvent peak.

Ethylene glycol $HOCH_2CH_2OH$ [Ethylene glycol (glycol), Special Class]

Ethylene glycol for Karl Fischer titration See ethylene glycol, for Karl Fischer titration.

Ethylene glycol, for Karl Fischer method Distill ethylene glycol, and collect the fraction at 195 °C to 198 °C. The water content is NMT 1.0 mg per mL.

Ethylenediamine $C_2H_8N_2$ [See monograph, Part II]

Ethylenediamine TS To 70 g of ethylenediamine, add 30 g of water.

Ethylenediaminetetraacetic acid disodium copper salt See ethylenediaminetetraacetic acid disodium copper salt tetrahydrate.

Ethylenediaminetetraacetic acid disodium copper salt tetrahydrate $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$ Blue powder, pH 7.0 to 9.0.

Purity *Clarity and color of solution*: Add 0.1 g of Ethylenediaminetetraacetic acid disodium copper salt tetrahydrate to 10 mL of freshly boiled and cooled water: the solution is blue in color and clear.

Content: NLT 98.0%.

Assay: Weigh accurately about 0.45 g of ethylenediaminetetraacetic acid disodium copper salt tetrahydrate and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH to about 1.5 by adding 100 mL of water and dilute nitric acid, add 5 mL of a solution of 1,10-phenanthroline monohydrate in methanol (1 in 20), and titrate with 0.01 mol/L bismuth nitrate solution (indicator: 2 drops of xylenol orange TS). The endpoint of the titration is when the yellow color of the solution turns to red.

Each mL of 0.01 mol/L bismuth nitrate solution
= 4.698 mg $C_{10}H_{12}CuN_2Na$

Ethylenediaminetetraacetic acid disodium salt See ethylenediaminetetraacetic acid disodium salt dihydrate.

Ethylenediaminetetraacetic acid disodium salt dihydrate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ [Special Class]

Ethylenediaminetetraacetic acid disodium salt dihydrate TS, 0.1 mol/L Dissolve 37.2 g of ethylenediaminetetraacetic acid disodium salt dihydrate in water to make 1000 mL.

Ethylenediaminetetraacetic acid disodium salt dihydrate TS, 0.4 mol/L, pH 8.5 Dissolve 148.9 g of ethylenediaminetetraacetic acid disodium salt dihydrate in water, adjust the pH to 8.5 with 8 mol/L sodium hydroxide solution, and add water to make 1000 mL.

Ethylenediaminetetraacetic acid disodium salt TS, 0.1 mol/L See ethylenediaminetetraacetic acid disodium salt TS, 0.1 mol/L.

Ethylenediaminetetraacetic acid disodium salt TS, 0.4 mol/L See ethylenediaminetetraacetic acid disodium salt TS, 0.4 mol/L.

Ethylenediaminetetraacetic acid disodium zinc salt See ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate.

Ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate $C_{10}H_{12}ZnN_2Na_2O_8 \cdot 4H_2O$ White powder.

The pH of ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate in aqueous solution (1 in 100) is 6.0 to 9.0.

Purity *Clarity and color of solution*: Dissolve 0.10 g of Ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

Content: NLT 98.0%

Assay: Weigh accurately about 0.5 g of ethylenediaminetetraacetic acid disodium zinc salt tetrahydrate and dissolve in water to make exactly 100 mL. To 10 mL of this solution, add 80 mL of water and dilute nitric acid to adjust the pH to about 2, and titrate with 0.01 mol/L bismuth nitrate solution (indicator: 2 drops of xylenol orange TS). The endpoint of the titration is when the yellow color of the solution turns to red.

(Ethyleneoxy)ethanol for gas chromatography Prepared for gas chromatography.

2-Ethylhexanoic acid $C_8H_{16}O_2$ A colorless liquid.

2-Ethylhexanoic acid See 2-ethylhexanoic acid.

***N*-Ethylmaleimide** $C_6H_7NO_2$ White crystals, having an irritating, characteristic odor. Freely soluble in ethanol (95), and slightly soluble in water.

Melting point: 43 ℃ to 46 ℃

Clarity and color of solution: The solution is clear and colorless (1 g, ethanol (95), 20 mL).

Content: NLT 99.0%.

Assay: Dissolve about 0.1 g of *N*-Ethylmaleimide, accurately weighed, in 20 mL of ethanol (95), add exactly 20 mL of 0.1 mol/L sodium hydroxide solution, and titrate with 0.1 mol/L hydrochloric acid (indicator: 2 drops of phenolphthalein TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide
= 12.513 mg $C_6H_7NO_2$

N-Ethylpiperidine benzylpenicillin Use the precipitate taken by filtration performed according to the Penicillin G Potassium Assay under Penicillin G Potassium. For the test solution used in this case, N-ethylpiperidine benzylpenicillin does not need to be added. Also, preserve the precipitate quantified from the test for later use.

***N*-Ethylpiperidine TS for penicillin** See *N*-Ethylpiperidine TS, for penicillin.

***N*-Ethylpiperidine TS, for penicillin** To an appropriate amount of N-ethylpiperidine, add the 4-fold volume of amyl acetate, blend, and then add n-ethylpiperidine benzylpenicillin to make a saturated solution. Store this solution at 0 °C to 8°C and use it within 3 days. Perform the assay of penicillin G sodium RS; the content of penicillin G sodium NLT 97% can be obtained . For use in testing, take it by absorbing, with a cotton stopper fit to the end of a pipet.

N-Ethylpiperidine $C_7H_{15}N$

Etilefrine hydrochloride $C_{10}H_{15}NO_2 \cdot HCl$ [See monograph]

Euglobulin TS (Prepare before use) Dissolve dried human plasma obtained from 20 mL of human serum in 200 mL of water and add acetic acid solution (1 in 50) to adjust the pH to 5.2 to 5.3. Centrifuge it at 2000 to 3000 rpm for 10 minutes, discard the clear supernatant, dissolve the precipitate in 20 mL of 0.067 mol/L phosphate buffer solution (pH 7.4), and filter. Store at 2 ℃ to 8 ℃.

Fast blue B salt $C_{14}H_{12}N_4O_2 \cdot ZnCl_4$: 475.47

Fehling's TS When using this solution, mix equal amounts of copper solution and alkaline tartrate solution.

Copper solution: Dissolve 34.66 g of copper(II) sulfate pentahydrate in water to make 500 mL. Keep this solution in well-filled, glass-stoppered bottles.

Alkaline tartrate solution: Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make 500 mL. Preserve in polyethylene bottles.

Fehling's TS for starch digestion test See Fehling's TS, for starch digestion test.

Fehling's TS, for starch digestion test When using this solution, mix equal amounts of copper solution and alkaline tartrate solution.

Copper solution: Weigh accurately 34.660 g of copper(II) sulfate pentahydrate, and dissolve in water to make exactly 500 mL. Keep this solution in well-filled, glass-stoppered bottles.

Alkaline tartrate solution: Dissolve 173 g of Potassium sodium tartrate tetrahydrate and 50 g of sodiumhydroxide in water to make exactly 500 mL. Preserve in polyethylene bottles.

Ferric chloride See Iron(III) chloride hexahydrate.

Ferric chloride solution Dissolve ferrous chloride 10 g with methanol to make 1 mL (doxazosin mesilate).

Ferric chloride TS See Iron(III) chloride TS.

Ferric chloride TS Dissolve 77 mg of Iron(III) chloride hexahydrate in 50 mL of acetic acid (96), slowly add 50 mL of sulfuric acid while cooling and mix (amorphous aescin and thiocolchicoside tablets).

Ferric chloride TS, dilute See Iron(III) chloride TS, dilute.

Ferric chloride-acetic acid TS See Iron(III) chloride-acetic acid TS.

Ferric chloride-iodine TS See Iron(III) chloride-iodine TS.

Ferric chloride-methanol TS See Iron(III) chloride-methanol TS.

Ferric chloride-potassium hexacyanoferrate(III) TS See Iron(III) chloride-potassium hexacyanoferrate(III) TS.

Ferric nitrate See iron(III) nitrate nonahydrate.

Ferric nitrate solution Dissolve 0.1 g of ferric nitrate in water to make 100 mL (ciclopirox olamine).

Ferric nitrate TS See Iron(III) nitrate TS.

Ferric perchlorate See iron(III) perchlorate hexahydrate.

Ferric perchlorate-anhydrous ethanol TS See iron(III) perchlorate-ethanol (99.5) TS.

Ferric sulfate See iron(III) sulfate *n*-hydrate.

Ferric sulfate TS See iron(III) sulfate TS.

Ferring's alkaline copper TS (alkaline copper TS of Ferring's TS) Weigh 4.0 g of copper(II) sulfate pentahydrate, 24 g of anhydrous sodium carbonate, 16 g of sodium bicarbonate, 180 g of anhydrous sodium sulfate, and 12 g of potassium sodium tartrate tetrahydrate, and add water to make 900 mL. Boil this solution for 10 minutes, cool, add water to make 1L, seal and allow to stand for 1 week. Then filter through a glass filter (G3), and preserve in light-resistant containers.

Ferroin TS Dissolve 0.7 g of ferrous sulfate heptahydrate and 1.76 g of o-phenanthroline hydrochloride monohydrate in water to make 100 mL.

Ferrous sulfate See iron(II) sulfate heptahydrate

Ferrous sulfate TS See iron(II) sulfate TS

Ferrous tartrate TS See iron(II) tartrate TS.

Fibrinogen A white, amorphous substance. Add 1 mL of isotonic sodium chloride injection to 10 mg of Fibrinogen. When warmed to 37 °C, it dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin. Fibrinogen is prepared from human or bovine blood by fractional precipitation with ethanol or ammonium sulfate. Fibrinogen may contain citrate, oxalate, and sodium chloride.

Fibrinogen Complies with dried human plasma fibrinogen standards under the specification for biological products (Purified Urokinase)

Fibrinogen solution Dissolve 1 g/bottle of dried fibrinogen in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.2), and adjust the pH to 7.2 (Purified Urokinase).

Fibrinogen TS Usually, to 0.1 g of fibrinogen, add 40 mL of 0.067 mol/L phosphate buffer solution (pH 7.4), and then filter. When determining the dissolution time according to the potency test of streptokinase, adjust the concentration of fibrinogen so that the dissolution time of the streptokinase RS (1 in 500) is within the range of 250 to 450 seconds. (Prepare before use.) (Streptokinase and Streptodornase)

Fluocinolone acetonide $C_{24}H_{30}F_2O_6$ [See monograph, Part I]

9-Fluorenylmethyl chloroformate $C_{15}H_{11}ClO_2$ [Special class]

9-Fluorenylmethyl chloroformate TS Weigh 250 mg of 9-fluorenylmethyl chloroformate, and dissolve in 250 mL of acetonitrile (sodium alendronate hydrate).

Fluorescein $C_{20}H_{12}O_5$ [Special class]

Fluorescein sodium $C_{20}H_{10}Na_2O_5$ [See monograph, Part I]

Fluorescein sodium TS Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

1-Fluoro-2,4-dinitrobenzene $C_6H_3(NO_2)_2F$ [Special class]

1-Fluoro-2,4-dinitrobenzene TS Dissolve 1-fluoro-2,4-dinitrobenzene in alcohol to obtain a concentration of 10 mg/mL (if not refrigerated, use within 5 days).

Folic acid $C_{19}H_{19}N_7O_6$ [See monograph, Part I on Folic Acid]

Folin TS Place 20 g of sodium tungstate (VI) dihydrate, 5 g of sodium molybdate dihydrate. and about 140 mL of water in a 300 mL volumetric flask, add 10 mL of diluted phosphoric acid (17 in 20) and 20 mL of hydrochloric acid, and boil gently using a reflux condenser with ground-glass joints for 10 hours. To the mixture, add 30 g of lithium sulfate and 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser. After cooling, add

water to make 200 mL, and filter through a glass filter. Store it free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

Formaldehyde solution HCHO [Special class].

Formaldehyde solution TS To 0.5 mL of formaldehyde solution, add water to make 100 mL.

Formaldehyde solution-sulfuric acid TS Add 1 drop of formaldehyde solution TS to 1 mL of sulfuric acid.

Formalin See Formaldehyde solution.

Formalin TS See Formaldehyde solution TS.

Formalin-sulfuric acid TS See Formaldehyde solution-sulfuric acid TS.

Formamide $HCONH_2$ [Special class]

Formamide for Karl Fischer titration See formamide, for Karl Fischer titration.

Formamide, for Karl Fischer titration $HCONH_2$ [Special class, the water content in 1 g of Formamide is NMT 1 mg.]

Formazine stock suspension Add 25 mL of hydrazinium sulfate TS to 25 mL of hexamine TS, and allow to stand at 25 ± 3 ℃ for 24 hours before use. This stock solution is effective for approximately 2 months in a glass container with no flaws on the surface. Shake well to mix before use.

Formic acid $CHNaO_2$ [Special class, density NLT 1.21 g/mL]

Formic acid buffer solution Dissolve 180 mL of formic acid, 100 mg of ammonium chloride, and 250 mL of ammonia water in 30 mL of water.

Formic acid, anhydrous CH2O2 A colorless, corrosive liquid, miscible with water and ethanol (95).

Specific gravity d_{20}^{20}: about 1.22

Content: NLT 98.0%

Assay: Weigh accurately 1 mL of formic acid, add 60 mL of water, and titrate with 1 mol/L sodium hydroxide VS. (Indicator: 0.5 mLof Phenolphthalein TS)

Each mL of 1 mol/L sodium hydroxide VS
= 46.03 mg of H2O2

Frede TS Dissolve 1 g of ammonium molybdate in 100 mL of sulfuric acid (cephalanthine).

Freund Complete Adjuvant A suspension of 5 mg of mycobacteria of *Corynebacterium butyricum*, killed by heating, in 10 mL of a mixture of 85 volumes of mineral oil and 15 volumes of Aracel A.

Fructose $C_6H_{12}O_6$ [See monograph, Part I]

Fuchsin A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95).

Loss on drying: 17.5 to 20.0% (1 g, 105 °C, 4 hours).

Residue on ignition: NMT 0.1%

Fuchsin TS, decolorized Dissolve 1 g fuchsin TS in 100 mL of water, warm the solution to about 50 ℃, and then cool with occasional shaking to mix. Allow this solution to stand for 48 hours, mix, and filter. To 4 mL of the filtrate, add 6 mL of dilute hydrochloric acid and water to make 100 mL. Allow the solution to stand for NLT 1 hour before use. Prepare before use.

Fuchsin TS, decolorized See decolorized Fuchsin TS.

Fuchsin-ethanol TS Dissolve 11 g of fuchsin in 100 mL of ethanol (95).

Fuchsin-sulfurous acid TS Dissolve 0.2 g of fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution prepared by dissolving 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid, and water to make 200 mL. Allow to stand for at least 1 hour. Prepare before use.

Fuming nitric acid See nitric acid, fuming.

Fuming sulfuric acid See sulfuric acid, fuming.

Furfural $C_5H_4O_2$ [Special class]

Furfural acetic acid TS Dissolve 100 mL of furfural in 2.5 mL of acetic acid (100). Store in a light resistant container with a stopper.

Furfural solution Freshly distilled furfural (discard 10% of initial distillate and 20% of residue). Dissolve 1 mL in water to make 100 mL. Prepare before use.

***D*-Galactosamine hydrochloride** $C_6H_{13}NO_5 \cdot HCl$ White powder.

Melting point: 180 ℃ (with decomposition)

Specific optical rotation $[\alpha]_D^{20}$: +90 to +97° [1 g, water, 100 mL, 100 mm]

Gauze [See monograph, Part II].

Gel type strongly acidic ion exchange resin for liquid chromatography (cross-linking rate: 6%) Prepared for liquid chromatography.

Gel type strongly acidic ion exchange resin for liquid chromatography (cross-linking rate: 8%) Prepared for liquid chromatography.

Gelatin [See monograph, Part II].

Gelatin peptone See peptone, gelatin.

Gelatin solution, 1% Dissolve 1 g of gelatin (pharmacopoeia) and 10 g of sodium chloride in water to make 100 mL and adjust the pH to 4.7 (policresulen).

Gelatin TS Dissolve 1 g of gelatin in 50 mL of water by gentle heating, and filter, if necessary. Prepare before use.

Gelatin TS, dilute Add a small amount of warm water to 5.0 g of gelatin (pharmacopoeia) for dissolution, add sodium chloride 10.0 g, potassium dihydrogen phosphate 13.6 g and thimerosal TS (1 in 100) 100 mL, add water for dilution, cool it, adjust the pH to 7.45 with 8 mol/L sodium hydroxide solution, and add purified water to make 1000 mL. Sterilize it (streptokinase and

streptodornase).

Gelatin-phosphate buffer solution Dissolve 13.6 g of potassium dihydrogen phosphate, 15.6 g of disodium hydrogen phosphate dodecahydrate, and 1.0 g of sodium azide in water to make 1000 mL, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75), and use this solution as Solution A. Dissolve 5.0 g of acid-treated gelatin in 400 mL of the Solution A by warming, cool, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75), and add the Solution A to make 1000 mL.

Gelatin-phosphate buffer solution, pH 7.0 Dissolve 1.15 g of sodium dihydrogen phosphate dihydrate, 5.96 g of disodium hydrogen phosphate dodecahydrate and 5.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g of gelatin to this solution by warming, and after cooling, add water to make 600 mL.

Gelatin-phosphate buffer solution, pH 7.4 To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution, add 39.50 mL of 0.2 mol/L sodium hydroxide TS and 50 mL of water. Add 0.2 g of gelatin to this solution, dissolve by warming, cool, adjust the pH to 7.4 with 0.2 mol/L sodium hydroxide TS, and add water again to make 200 mL.

Gelatin-tris buffer solution Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.22 g of sodium chloride in 700 mL of water. Separately, dissolve 10 g of acid-treated gelatin in 200 mL of water by warming. After cooling, combine the two solutions, adjust the pH to 8.8 with dilute hydrochloric acid, and add water to make 1000 mL.

Gelatin-tris buffer solution, pH 8.0 Dissolve 40 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 5.4 g of sodium chloride in 500 mL of water. Add 1.2 g of gelatin to this solution, dissolve by warming, cool, adjust the pH to 8.0 with dilute hydrochloric acid, and add water to make 600 mL.

Gelatin, acid-treated [See monograph, Part II Gelatin. Its isoelectric point is at pH between 7.0 and 9.0.]

Giemsa reagent Dissolve 3 g of azure II-eosin and 0.8 g of azure-II in 250 g of glycerol at 60 ℃ by heating, cool, then add 250 g of methanol, and mix well. Allow it to stand for 24 hours, and then filter. Preserve in a stoppered container.
Combine eosin and azure II to make azure II-eosin.
Azure II is a mixture of equal amounts of methylene azure (azure I, prepared by oxidizing methylene blue) and methylene blue.

Glass fiber See glass wool.

Glass wool [Special Class]

D-glucosamine hydrochloride $C_6H_{13}NO_5 \cdot HCl$, White crystal or crystalline powder.

Content: NLT 98%.

Assay: Weigh accurately about 0.4 g of D-glucosamine hydrochloride, dissolve in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 21.56 mg of $C_6H_{13}NO_5 \cdot HCl$

Glucose $C_6H_{12}O_6$ [See monograph, Part I]

Glucose TS Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

Glucose-peptone medium for sterility test See Glucose II Peptone Medium for Sterility under General Tests.

Glucose-peptone medium, for sterility test See glucose-peptone medium, for sterility test in the Sterility under the General Tests.

***D*-Glucuronic acid** $C_6H_{10}O_7$ [Special class]

Glutamic acid TS Dissolve 0.125 g of *L*-glutamic acid in acetic acid to make 100 mL. Prepare before use.

***L*-Glutamic acid** $HOOC(CH_2)_2CH(NH_2)COOH$ [Special class]

7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin
White powder. Freely soluble in acetic acid (100), sparingly soluble in dimethylsulfoxide and practically insoluble in water.

Absorbance $E_{1cm}^{1\%}$ (325 nm): 310 to 350 [2mg, diluted acetic acid (1 in 500), 200 mL]

Specific optical rotation $[\alpha]_D^{20}$: - 50 to - 60° [0.1 g, diluted acetic acid (1 in 2), 10 mL, 100 mm]

Purity *Related substances*: Dissolve 5 mg of 7-(glutarylglycyl-L-arginylamino)-4-methylcoumarin in 0.5 mL of acetic acid (31), and use this solution as the test solution. Perform the test with this solution as directed under the Thin Layer Chromatography. Spot 5 μL of the test solution on the thin plate made of silica gel for thin-layer chromatography. Next, develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (15 : 12 : 10 : 3) to a distance of about 10 cm, take out the plate, air-dry it, and then dry at 80 ℃ for 30 minutes. After cooling, place the plate in a container filled with iodine vapor and allow to stand for 30 minutes; no spots other than the principal spot with the R_f value of about 0.6 appear.

7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS Dissolve 5 mg of 7-(glutarylglycyl-*L*-arginylamino)-4-methylcoumarin in 0.5 to 1 mL of acetic acid (31), and freeze-dry it. Dissolve in 1 mL of dimethylsulfoxide, and use this solution as solution A. Dissolve 30.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.6 g sodium chloride in 400 mL of water, add dilute hydrochloric acid to adjust the pH to 8.5, and then add water to make 500 mL. Use this solution as solution B. Mix 1 mL of solution A with 500 mL of solution B before use.

Glycerin $C_3H_8O_3$ [Special class, See monograph, Part I on Concentrated Glycerin]

Glycine H_2NCH_2COOH [Special class]

Graphite carbon for gas chromatography Prepared for gas chromatography.

Griess-Romijin's nitric acid reagent Grind 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 1.5 g of zinc flour in a mortar and pestle. Preserve in light-resistant, tight containers.

Griess-Romijin's nitrous acid reagent Grind 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 89 g of L-tartaric acid in a mortar and pestle. Preserve in light-resistant, tight containers.

Guaiacol $CH_3OC_6H_4OH$, Colorless to yellowish, transparent

crystal or colorless crystal, having a characteristic aroma. Soluble in water and miscible with ether, ethanol (95) or chloroform.

Melting point: About 28 ℃

Purity: Perform the test with 0.5 μL of guaiacol as directed under the Gas Chromatography according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of guaiacol by the area percentage; it is NLT 99.0%.

Operating conditions

Detector: Flame ionization detector

Column: A glass column about 3 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography coated with polyethylene glycol 20 M for gas chromatography in the ratio of 20% (150 μm to 180 μm in particle diameter).

Column temperature: Constant temperature around 200 ℃.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of guaiacol is 4 to 6 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height obtained from 0.5 μL of Guaiacol is about 90% of the full scale.

Time span of measurement: About 3 times the retention time of guaiacol.

Gum arabic solution Add water to 200 g of very fine powder of gum arabic (Merck No. 4282) to make 2 000 mL. Centrifuge this solution and store aliquots of 330 mL at 20 ℃ (pancreas I).

Gum arabic, 10% To 20 g of gum arabic, add water to make 200 mL. Centrifuge this solution and take the clear supernatant to use (pancreatin II).

Gum solution, for preparing Stoke emulsion Dissolve 200 g of gum arabic in 2000 mL of water, stir for 2 hours, and centrifuge for about 30 minutes until the liquid becomes almost transparent (pancreas).

Hans TS To 20 g of iodine bromide, add 1000 mL of acetic acid (31) to dissolve. Place in a glass-stoppered bottle, and store in a cold place.

Helium He NLT 99.995 vol%.

Hematoxylin $C_{16}H_{14}O_6 \cdot nH_2O$ White or yellow to brown crystals or crystalline powder, soluble in warm water or ethanol (95), but slightly soluble in cold water.

Residue on ignition: NMT 0.1% (1 g).

Hematoxylin TS Dissolve 1 g of hematoxylin in 12 mL of ethanol (95). Separately, dissolve 20 g of aluminum potassium sulfate in 200 mL of warm water, cool, and filter. After 24 hours, mix these two prepared solutions, allow to stand for 8 hours in a wide-mouthed bottle without using a stopper, and filter.

Heparin sodium [See monograph, Part I].

Purity *Related substances*: To 0.2 g of 2-Ethylhexanoic Acid, add 5 mL of water, 3 mL of dilute hydrochloric acid and 5mL of hexane, shake to mix for 1 minute, allow to stand to separate the layers, and use the clear supernatant. Perform the test with 1 **μ**L of this solution as directed under the gas Chromatography according to the operating condition under 2-ethyl hexanoate Amoxicillin Sodium(Purity (6); the total area of the peak areas of individual related substances, excluding the solvent and major peak, is NMT 2.5% of the major peak area.

Hepatic extract Add 170 mL of distilled water to 10 g of hepatic powder, extract at 60 ℃ for 3 to 4 hours, and boil for 10 minutes. After cooling, adjust the pH to 6.5, and filter before use (lactobacillus bifidus).

***n*-Heptane** $CH_3(CH_2)_5CH_3$ [Special class]

Hexachloroplatinate(IV) hexahydrate $H_2PtCl_6 \cdot 6H_2O$ [Special class]

Hexachloroplatinate(IV) TS Dissolve 2.6 g of hexachloroplatinate(IV) hexahydrate in water to make 20 mL (0.125 mol/L).

Hexachloroplatinate(IV)-potassium iodide TS Add 97 mL of water and 100 mL of potassium iodide solution (3 in 50) to 3 mL of hexachloroplatinate(IV) TS. Prepare before use.

Hexamethylenetetramine $(CH_2)_6N_4$ [Special class]

Hexamine See Hexamethylenetetramine.

Hexamine TS Dissolve 2.5 g of hexamine in 25 mL of water.

Hexane C_6H_{14} [Special class]

Hexane for absorption spectroscopy [Special class] Determine the absorbance using water as the blank; it is NMT 0.10 at a wavelength of 220 nm and NMT 0.02 at a wavelength of 260 nm. Also, absorption cannot be seen at wavelengths of 260 to 350 nm.

***n*-Hexane for absorption spectroscopy** See hexane for absorption spectroscopy.

Hexane for liquid chromatography $CH_3(CH_2)_4CH_3$ A colorless, clear liquid. Miscible with ethanol, ether, chloroform and benzene.

Boiling point: About 69 ℃

Purity (1) *Ultraviolet absorbing substances:* Perform the test with Hexane for Liquid Chromatography as directed under the Ultraviolet-visible Spectroscopy, using water as the blank; the absorbance at 210 nm is NMT 0.3 and at 250 nm to 400 nm, NMT 0.01.

(2) *Peroxide:* To 20 g of Hexane for Liquid Chromatography, add a solution previously prepared by adding 25 mL of potassium iodide solution (1 in 10) to 100 mL of water and 25 mL of dilute sulfuric acid. Shake well to mix, and allow to stand for 15 minutes in a dark place. Titrate this solution, while shaking well, with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank test in the same manner and make any necessary correction (NMT 0.0005%).

***n*-hexane for liquid chromatography** See hexane for liquid chromatography.

Hexyl silyl silica gel for liquid chromatography Prepared for liquid chromatography.

***L*-histidine hydrochloride monohydrate** $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ [Special class]

L-Histidine hydrochloride See L-histidine hydrochloride monohydrate.

Honokiol $C_{18}H_{18}O_2 \cdot xH_2O$ Odorless white, crystals or crystalline powder.

Purity: Dissolve 1 mg of Honokiol in the mobile phase to make exactly 10 mL, and use this solution as the test solution. Perform the liquid chromatography with 10 μL of this solution as directed in the Assay under Magnolia bark. Total area of peaks other than honokiol from the test solution is not larger than one tenth of total area of the peaks other than the solvent peak.

Horse serum Collect the blood from a horse in a flask, coagulate blood, and allow to stand at ordinary temperature until the serum is separated. Transfer the separated serum in a glass container, and cryopreserve at -20 ℃.

Human antithrombin III A serine lyase inhibitor obtained from healthy human serum. It is a protein that inhibits the activity of blood coagulation X factor and thrombin. Each mg of human antithrombin III contains NLT 300 units. However, 1 unit of human antithrombin III is the amount of protein that inhibits 1 unit of thrombin at 25℃ in the presece of heparin.

Hyaluronidase solution Take hyaluronidase derived from Streptomyces hyalurolyticus, dissolve in isotonic sodium chloride injection to 100 turbidity reducing units per mL (sodium hyaluronate, sodium hyaluronate ocular injection).

Hydralazine hydrochloride $C_8H_8N_4 \cdot HCl$ [See monograph, Part I]

Hydrazine dihydrochloride $(NH_2)_2 \cdot 2HCl$ A white powder

Content: NLT 98.0%

Assay: Take exactly 34 mg of hydrazine dihydrochloride, and add it to 50 mL of water. Dissolve carefully while stirring, and add 1 g of sodium bicarbonate. Quantify with 0.1 mol/L iodine solution VS, according to the potentiometric titration.

Each mL of 0.1 mol/L Iodine VS
= 2.36 mg of Hydrazine dihydrochloride ($(NH_2)_2 \cdot 2HCl$ 104.97)

Hydrazine hydrate See Hydrazine mono hydrate.

Hydrazine monohydrate $H_2NNH_2 \cdot H_2O$ A colorless liquid, having a characteristic odor.

Hydrazine sulfate See hydrazinium sulfate.

Hydrazine sulfate TS See hydrazinium sulfate TS.

Hydrazine TS Add a mixture of 2 mol/L sodium hydroxide TS and methanol (1 : 1) to 10.0 mg of hydrazine sulfate to make 50 mL. Take exactly an appropriate amount of this solution to make a solution containing 2.0 μg of hydrazine sulfate per each mL.

Hydrazinium sulfate $N_2H_6SO_4$ [Special class]

Hydrazinium sulfate TS Dissolve 2 g of hydrazinium sulfate and 6.5 g of sodium acetate in water to make 100 mL.

Hydrazinium sulfate TS Dissolve 1.0 g of hydrazinum sulfate in water to make 100 mL.

Hydrobromic acid HBr [Special class].

Hydrochloric acid HCl [Special Class]

Hydrochloric acid TS for bacterial endotoxins test, 0.1 mol/L See hydrochloric acid TS, 0.1 mol/L, for bacterial endotoxins test.

Hydrochloric acid TS, 0.001 mol/L Add water to 10 mL of 0.1 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.01 mol/L Add water to 100 mL of 0.1 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.02 mol/L Add water to 100 mL of 0.2 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.05 mol/L Add water to 100 mL of 0.5 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.06 mol/L Add water to 60 mL of 1 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.1 mol/L Add water to 100 mL of 1 mol/L hydrochloric acid TS to make 1000 mL.

Hydrochloric acid TS, 0.1mol/L, for bacterial endotoxins test To 9.0 mL of hydrochloric acid for bacterial endotoxins test, add water for bacterial endotoxins test to make 1000 mL.

Hydrochloric acid TS, 0.2 mol/L To 18 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 0.5 mol/L To 45 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 1 mol/L To 90 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 1.2 mol/L To 108 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 2 mol/L To 180 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 3 mol/L To 270 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 5 mol/L To 450 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 6 mol/L To 540 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 7.5 mol/L To 675 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid TS, 9 mol/L To 810 mL of hydrochloric acid, add water to make 1000 mL.

Hydrochloric acid-ammonium acetate buffer solution, pH 3.5 Dissolve 25 g of ammonium acetate in 45 mL of 6 mol/L hydrochloric acid TS 45 and add water to make 100 mL.

Hydrochloric acid-ethanol (95) TS Dilute 23.6 mL of hydrochloric acid with ethanol (95) to make 100 mL.

Hydrochloric acid-ethanol solution Add 99% ethanol to 5.0

mL of 0.1 mol/L hydrochloric acid to make 1 L (isotretinoin).

Hydrochloric acid-ethanol TS See hydrochloric acid-ethanol (95) TS.

Hydrochloric acid-methanol TS Take 1 mL of 1 mol/L hydrochloric acid TS and add methanol to make 100 mL.

Hydrochloric acid-methanol TS, 0.01 mol/L Add methanol to 47.5 mL of hydrochloric acid to make 1000 mL, and to a suitable volume of it, add methanol to make exactly 10-fold dilution. To an appropriate amount of this solution, add methanol for 5-fold dilution.

Hydrochloric acid-methanol TS, 0.01 mol/L Add methanol to 0.05 mol/L methanolic hydrochloride TS for exact 5-fold dilution.

Hydrochloric acid-methanol TS, 0.05 mol/L Add methanol to 47.5 mL of hydrochloric acid to make 1000 mL, and to a suitable volume of it, add methanol for exact 10-fold dilution.

Hydrochloric acid-methanol TS, 0.1 mol/L Add methanol to 47.5 mL of hydrochloric acid to make 1000 mL, and to a suitable volume of it, add methanol to make exactly 5-fold dilution.

Hydrochloric acid-potassium chloride buffer solution, pH 2.0 Add 88.0 mL of 0.2 mol/L potassium chloride TS to 10.0 mL of 0.2 mol/L hydrochloric acid, adjust the pH to 2.0 ± 0.1 by further adding 0.2 mol/L hydrochloric acid, and then add water to make 200 mL.

Hydrochloric acid-sodium chloride buffer solution, pH 1.2 Place 2 g of sodium chloride in a 1-L volumetric flask, and add 80 mL of 1 mol/L hydrochloric acid and then water to make 1 L (Emepronium bromide).

Hydrochloric acid, dilute Add water to 23.6 mL of hydrochloric acid to make 100 mL (10%).

Hydrochloric acid, purified Add 0.3 g of potassium permanganate in 1000 mL diluted hydrochloric acid (1 in 2), distill, discard the first 250 mL of the distillate and then take the subsequent 500 mL of the distillate.

Hydrochloric acid-methanol TS, 0.1 mol/L Add methanol to 47.5 mL of hydrochloric acid to make 1000 mL, and to a suitable volume of it, add methanol to make exactly 5-fold dilution.

Hydrocortisone $C_{21}H_{30}O_5$ [See monograph, Part I]

Hydrocortisone acetate $C_{23}H_{32}O_6$ [See monograph, Part I]

Hydrocortisone acetate $C_{23}H_{32}O_6$ [See monograph, Part II on Hydrocortisone Acetate]

Hydrofluoric acid HF [Special class].

Hydrogen H_2 [Reference material] NLT 99.99%.

Hydrogen chloride-ethanol (99.5) TS Slowly add 100 mL of sulfuric acid dropwise to 100 mL of hydrochloric acid, dry the hydrogen chloride obtained therefrom in a wash bottle containing sulfuric acid, and pass the result through 75 g of ethanol (99.5) cooled in an ice bath, until the increase in mass reaches 25 g. Prepare before use.

Hydrogen chloride-ethanol TS See hydrogen chloride-ethanol (99.5) TS.

Hydrogen peroxide (30) H_2O_2 [Special class, Concentration: 30.0 to 35.5%]

Hydrogen peroxide TS To 1 volume of hydrogen peroxide (30), add 9 volumes of water. Prepare before use (3%).

Hydrogen peroxide TS, dilute To 1 mL of hydrogen peroxide (30), add 500 mL of water, mix, take 5 mL of this solution, and add water to make 100 mL. Prepare before use.

Hydrogen peroxide-sodium hydroxide TS To a mixture of water and hydrogen peroxide (30) (9 : 1), add 3 drops of bromophenol blue TS, and then add 0.01 mol/L sodium hydroxide TS until the color of the solution becomes bluish purple. Prepare before use.

Hydrogen peroxide, strong See hydrogen peroxide (30).

Hydrogen sulfide H_2S A colorless, poisonous gas, heavier than air. It dissolves in water. Prepare by treating iron(II) sulfide with dilute sulfuric acid or dilute hydrochloric acid. Any sulfides other than Iron(II) sulfide may be used if they yield hydrogen sulfide with dilute acids.

Hydrogen sulfide TS A saturated solution of hydrogen sulfide. Prepare by passing hydrogen sulfide into cold water. Preserve in well-filled, light-resistant bottles, in a dark, cold place.

Hydrogen tetrachloroaurate(III) tetrahydrate $HAuCl_4 \cdot 4H_2O$ [Special Class]

Hydrogen tetrachloroaurate(III) TS Dissolve 1 g of hydrogen tetrachloroaurate(III) tetrahydrate in 35 mL of water.

Hydroiodic acid HI [Special Class]

Hydroiodic acid TS Dissolve 0.5 g of sodium iodide in water to make 100 mL, and add 10 mL of hydrochloric acid. Prepare before use

Hydroquinone $C_6H_4(OH)_2$ [Hydroquinone (1, 4-benzenediol), Special class]

Hydroquinone TS, 1% Dissolve 1 g of hydroquinone in 100 mLdp of water, add 1 drop of sulfuric acid, and shake to mix. Prepare before use.

Hydroxy Naphthol Blue $C_{20}H_{14}N_2O_{11}S_3$ [Special class] Deposited on crystals of sodium chloride in the concentration of about 1%. Hydroxy Naphthol Blue appears as blue, fine crystals. Hydroxy Naphthol Blue is very soluble in water. At pH 12 to 13, Hydroxy Naphthol Blue shows a pink color with existence of calcium ion, and a deep blue color with existence of ethylenediaminetetraacetic acid disodium dihydrate.

Sensitivity for Quantiative Test of Calcium: Dissolve 0.3 g of Hydroxy Naphthol Blue in 100 mL of water, add 10 mL of sodium hydroxide TS, add 1 mL of a solution of calcium chloride dihydrate (1 in 200), dilute with water to make 165 mL: a pink

color develops. To this solution, add 1.0 mL of 0.05 mol/L disodium dihydrogen ethylendiamine tetraacetate VS: the color changes to deep blue.

Hydroxy propylsilyl silca gel for liquid chromatography Prepared for liquid chromatography.

2-Hydroxy-1-(2'-hydroxy-4'-sulfone-1'-naphthylazo)-3-naphthoic acid $C_{21}H_{14}N_2O_7S$ [Special class]

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[-2(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzodiazepine-4(5H)-one hydrochloride** $C_{20}H_{24}N_2O_3S \cdot HCl$ White crystals or crystalline powder, having a slight, characteristic odor.

Method of preparation: Add 50 mL of ethanol (99.5) to 9 g of diltiazem hydrochloride, and heat to 80 °C to dissolve. Slowly add 50 mL of ethanol (99.5) (33 in 500) of potassium hydroxide dropwise to this solution and heat while shaking to mix for 4 hours. After cooling, filter and evaporate the filtrate to dryness. Dissolve the residue in ethanol (99.5). Slowly add ethanol (99.5) solution of hydrochloric acid (59 in 250) to make it acidic, and filter it. Slowly add ether to the filtrate, filter and dissolve the resulting crystals, add 0.5 g of activated charcoal, allow to stand, and then filter. Cool the filtrate in an ice-methanol bath. Filter the resulting crystals, and wash with ethanol (99.5). Add ethanol (99.5) again, and heat to dissolve. After cooling, filter the resulting crystals, and dry in vacuum.

Purity: Weigh 50 mg of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[-2(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzodiazepine-4(5H)-one hydrochloride, dissolve in chloroform to make exactly 10 mL, and use this solution as the test solution. With this solution, perform the test as directed under the Thin Layer Chromatography. Spot 20 μL of the test solution onto a thin layer plate made of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), chloroform, water and acetic acid (100) (12 : 10 : 3 : 2) to a distance of about 13 cm, and air-dry the plate. Spray evenly Iodine TS on the plate; no spots other than the principal spot are observed.

Water: NMT 1.0% (0.5 g).

Content: NLT 99.0%, calculated on the anhydrous basis.

Assay: Weigh accurately about 0.5 g of *d*-3-Hydroxy-*cis*-2,3-dihydro-5-[-2(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzodiazepine-4(5H)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.89 mg of $C_{20}H_{24}N_2O_3S \cdot HCl$

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[-2(dimethylamino)ethyl]-2-*(p*-methoxyphenyl)-1,5-benzodiazepine-4(5H)-one hydrochloride** See *d*-3-Hydroxy-*cis*-2,3-dihydro-5-[-2(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzodiazepine-4(5H)-one hydrochloride.

***m*-Hydroxyacetophenone** $C_8H_8O_2S$ White to pale yellow crystalline powder

Melting point: About 96°C

Purity *Related substances:* Perform the test with 10 μL of a solution of m-Hydroxyacetophenone in 0.1 mol/L phosphate buffer solution in methanol at pH 4.5 (1 in 200) as directed in the Assay under Cefalexin; Any obstructive peaks for determination of Cefalexin are not observed.

***p*-Hydroxyacetophenone** $C_8H_8O_2$ White to pale yellow crystals or crystalline powder. Freely soluble in methanol.

Melting point: 107 111 ℃

Purity: Weigh about 1 mg of *p*-hydroxyacetophenone, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Perform the test with 20 μL of this solution as directed under the Liquid Chromatography according to the Assay under Peony Root. The total area of the peaks other than the peak of *p*-hydroxyacetophenone in the test solution is not larger than 0.03% of the total area of the peaks other than the solvent peak.

Benzyl *p*-hydroxybenzoic acid See benzyl parahydroxybenzoate.

Isopropyl *p*-hydroxybenzoic acid See isopropyl p-hydroxybenzoate.

***p*-Hydroxybenzoic acid** See parahydoroxybenzoic acid.

***p*-Hydroxybenzoic acid** $C_7H_6O_3$, white crystals.

Melting point: between 212 and 216 ℃

Content: NLT 98.0%

Assay: Weigh accurately about 0.7 g of p-hydroxybenzoic Acid, dissolve in 50 mL of acetone, add 100mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 69.06 mg of $C_7H_6O_3$

2-Hydroxybenzyl Alcohol $C_7H_8O_2$ A rhubarb white plate. 2-Hydroxybenzyl Alcohol is very soluble in ethanol, chloroform or ether, and soluble in water or benzene.

Assay: Perform the test with the appropriate test solution of 2-Hydroxybenzyl Alcohol as directed under the Gas Chromatography according to the following conditions. The peak area of 2-hydroxybenzyl alcohol is NLT 99.0% of the total peak area.

Operating conditions

Detector: Flame ionization detector

Column: The inner wall of a capillary column with an internal diameter of about 0.25 mm and a length of about 30 m, coated with dimethyl polysiloxane gum for gas chromatography to a thickness of about 1 μm.

Injector temperature: 250 ℃

Column temperature: Hold at 150 ℃, then raise the temperature by 10 ℃ per minute to 280 ℃.

Detector temperature: 300 ℃

Carrier gas: Helium

Melting point: 83 to 85℃

1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol $C_3H_6N_4OS$ White, crystals or powder.

Melting point: 136 to 141 ℃

Purity *Related substances*: Dissolve 0.10 g of 1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol in 1 mL of water, and use this solution as the test solution. Pipet 0.5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. With these solutions, perform the test as directed under the Thin Layer Chromatography. Spot 1 □L each of the test solution and the standard solution on the thin plate made of silica gel for thin-layer chromatography (with fluorescent agent). Develop with a mixture of ethyl acetate, water, methanol and formic

acid (60 : 10 : 7 : 6) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot obtained from the test solution are not more intense than those from the diluted standard solution

***N*2-Hydroxyethylpiperazine *N*′2-ethanesulfonic acid** $C_8H_{18}N_2O_4S$ A white crystalline powder.

Purity *Clarity and color of solution*: Dissolve 11.9 g of *N*2-hydroxyethylpiperazine *N*′2-ethanesulfonic acid in 50 mL of water; the solution is clear and colorless.

Content: NLT 99.0%

Assay: Weigh accurately about 1 g of *N*2-hydroxyethylpiperazine *N*′2-ethanesulfonic acid, dissolve in 60 mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.5 mol/L sodium hydroxide VS
= 119.15 mg of $C_8H_{18}N_2O_4S$

4-Hydroxyisophthalic acid $HOC_6H_3(COOH)_2$ White, crystals or crystalline powder

Content: NLT 98.0%

Assay: Take exactly 0.14 g of 4-Hydroxyisophthalic acid and add it to 50 mL of ethanol (95). Perform quantification using 0.1 mol/L sodium hydroxide TS according to the potentiometric titration.

0.1mol/L sodium hydroxide TS
= 9.106 mg of $HOC_6H_3(COOH)_2$

Hydroxylamine hydrochloride $NH_2OH{\cdot}HCl$ [Special class]

Hydroxylamine hydrochloride See hydroxylammonium chloride.

Hydroxylamine hydrochloride solution See hydroxylamine hydrochloride solution.

Hydroxylamine hydrochloride TS Blend hydroxylamine hydrochloride solution (34.8 in 100), sodium acetate trihydrate, 1 mol/L sodium hydroxide TS and ethanol (1 : 1 : 4).

Hydroxylamine hydrochloride TS Blend hydroxylammonium chloride solution (34.8 in100) with a mixture of sodium acetate trihydrate,·1 mol/L-sodium hydroxide TS and ethanol (1 : 1 : 4).

Hydroxylamine hydrochloride TS See hydroxylammonium chloride TS.

Hydroxylamine hydrochloride TS, pH 3.1 See hydroxylammonium chloride TS, pH 3.1.

Hydroxylamine hydrochloride-ethanol TS See hydroxylamine hydrochloride-ethanol TS.

Hydroxylamine hydrochloride-ferrous chloride TS See hydroxylammonium chloride-iron(II) chloride TS

Hydroxylamine hydrochloride-iron(II) chloride TS Add hydrochloric acid to 100 mL of ethanol (95) solution of iron(II) hydrochloride (1 in 200) to make it acidic, then dissolve in 1 g of hydroxylamine hydrochloride.

Hydroxylamine perchlorate $NH_2OH{\cdot}HClO_4$ Hygroscopic, white crystals. Soluble in water or ethanol (95). Melting point: Between 87.5℃ and 90℃.

Hydroxylamine perchlorate TS Ethanol (99.5) A solution containing 13.4% of hydroxylamine perchlorate. Preserve in tight containers in a cold place.

Hydroxylamine perchlorate-anhydrous ethanol TS See hydroxylamine perchlorate-ethanol (99.5) TS.

Hydroxylamine perchlorate-ethanol (99.5) TS Add ethanol (99.5) to 2.99 mL of hydroxylamine perchlorate to make 100 mL. Preserve in tight containers in a cold place.

Hydroxylamine TS Dissolve 10 g of hydroxylammonium chloride in 20 mL of water, and add ethanol (95) to make 200 mL. To this solution, add 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, while stirring, and filter. Prepare before use.

Hydroxylamine TS, alcoholic Dissolve 3.5 g of hydroxylamine in 95 mL of ethanol (60%) add 0.5 mL of a solution of methyl orange in ethanol (60%)) and 0.5 mol/L potassium hydroxide in ethanol (60% of ethanol) to give a pale yellow color. Dilute to 100 mL with ethanol (60%).

Hydroxylamine TS, alkaline Mix equal volumes of a solution of hydroxylammonium chloride in methanol (7 in 100) and a solution of sodium hydroxide in methanol (3 in 25), and filter. Prepare before use.

Hydroxylamine TS, neutral Mix equal volumes of hydroxylammonium chloride solution and buffer solution, and adjust the pH to 7.0 ± 0.1. To 1 volume of this neutral solution, add 8 volumes of water and 2 volumes of 95% ethanol. This solution should be used within 1 day (cefacetrile sodium, fibracillin).

Hydroxylamine-acetate TS See hydroxylammonium chloride-acetate TS.

Hydroxylammonium chloride $NH_2OH{\cdot}HCl$ [Special class]

Hydroxylammonium chloride solution Weigh 350 g of hydroxylammonium chloride and dissolve in water to make 1000 mL (cefacetrile sodium, fibracillin).

Hydroxylammonium chloride TS Dissolve 20 g of hydroxylammonium chloride in water to make 65 mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia water (28) until the solution exhibits a yellow color. Add 10 mL of a solution of sodium diethyldithiocarbamate trihydrate (1 in 25) again, shake well to mix, and allow to stand for 5 minutes. Extract this solution with 10 to 15 mL of chloroform, add 5 drops of copper sulfate solution (1 in 100) to 5 mL of the extract, shake to mix, and repeat the extraction until the yellow color of the solution disappears. Add 1 to 2 drops of thymol blue TS, add dropwise dilute hydrochloric acid to this water layer until it exhibits a red color, then add water to make 100 mL.

Hydroxylammonium chloride TS, pH 3.1 Dissolve 6.9 g of hydroxylammonium chloride in 80 mL of water, add dilute sodium hydroxide TS to adjust the pH to 3.1, and add water to make 100 mL.

Hydroxylammonium chloride-acetate TS Mix hydroxylammonium hydrochloride solution (34.8 in 100) with sodium acetate-sodium hydroxide TS and water (1 : 2 : 2), and adjust to pH 6.9 to 7.1. Prepare before use.

Hydroxylammonium chloride-ethanol TS To prepare it, mix 1 volume of hydroxylammonium hydrochloride solution (34.8 in 100), 1 volume of sodium acetate-sodium hydroxide TS, and 4 volumes of ethanol.

Hydroxylammonium chloride-ferrous chloride TS See Hydroxylammonium chloride-iron(II) chloride TS

Hydroxylammonium chloride-iron(II) chloride TS Add hydrochloric acid to 100 mL of ethanol (95) solution of iron(II) hydrochloride (1 in 200) to make it acidic, and dissolve in 1 g of hydroxyl ammonium hydrochloride.

***N*-(3-Hydroxyphenyl)acetamide** $C_8H_9NO_2$ White to pale yellowish white crystals. Freely soluble in ethanol (95), and sparingly soluble in water.

Melting point: 146 to 149 ℃

Purity *Clarity and color of solution*: Dissolve 0.5 g of *N*-(3-Hydroxyphenyl)acetamide in 50 mL of water; the solution is clear and colorless.

Related substances: Dissolve 0.1 g of *N*-(3-Hydroxyphenyl)acetamide in water to make 1000 mL. Pipet 10 mL of this solution, add 6.5 mL of acetonitirle and water to make exactly 50 mL, and use this solution as the test solution. Perform the test with 10 μL of this solution as directed in the Assay under Aspoxicillin Hydrate any peak other than those of of *N*-(3-Hydroxyphenyl)acetamide and the solvent does not appear.

3-*(p*-hydroxyphenyl)propionic acid $C_9H_{10}O_3$

Description: 3-*(p*-Hydroxyphenyl)propionic acid is white to pale yellowish brown crystals or crystalline powder, having a faint, characteristic odor.

Content: NLT 99.0%

Assay: Weigh accurately about 0.2 g of 3-*(p*-hydroxyphenyl)propionic Acid, previously dried (in vacuum 60 °C, 4 hours), dissolve in 5 mL of methanol, add 45 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 16.617 mg of $C_9H_{10}O_3$

8-Hydroxyquinoline C_9H_7NO [Special class]

Hypophosphoric acid H_3PO_2 [First Class]

Hypoxanthine $C_5H_4N_4O$ White crystals or crystalline powder. Soluble in ammonia TS, sparingly soluble in dilute hydrochloric acid or hot water, very slightly soluble in water, and practically insoluble in methanol.

Related substances: Dissolve 5.0 mg of Hypoxanthine in 100 mL of ammonia water (28) in methanol (1 in 10). Proceed with this solution as directed in the Purity under Mercaptopurine; any spot other than the principal spot with the R_f value of about 0.2 does not appear.

Content: 97.0 to 103.0%

Assay: Weigh accurately about 0.15 g of Hypoxanthine, previously dried at 105 °C for 3 hours, and dissolve in phosphate buffer solution (pH 7.0) to make exactly 1000 mL. Pipet 10 mL of this solution, and add phosphate buffer solution (pH 7.0) to make exactly 250 mL. Read the absorbance *A* of this solution at the wavelength of 250 nm using phosphate buffer solution (pH 7.0) as the blank.

Ibuprofen $C_2H_{18}N_2$ [See monograph, Part I]

Imidazole $C_3H_4N_2$ White to pale yellow crystals. Freely soluble in water.

Absorbance $E_{1cm}^{1\%}$ (313 nm) NMT 0.031 (8 g, water, 100 mL)

Melting point: 89 ℃ to 92 ℃

Imidazole buffer solution Dissolve 3.4 g of imidazole and 5.8 g of sodium chloride in 700 mL of purified water and add 15 mL of 1 mol/L hydrochloric acid to adjust the pH to 7.5. Add purified water to make 1 L. Sterilize (streptokinase and streptodornase).

Imidazole for Karl Fischer titration See the Water Determination under the General Test.

Imidazole TS Dissolve 8.25 g of imidazole with 65 mL of water, adjust the pH to 6.80 ± 0.05 with 5 mol/L hydrochloric acid TS, and add water to make exactly 100 mL.

Iminodibenzyl $C_{14}H_{13}N$ White to light brown crystals or crystalline powder, having a slight, characteristic odor.

Melting point: 104 ℃ to 110 ℃

Purity (1) *Clarity and color of solution*: Dissolve 1.0 g of Iminodibenzyl in 20 mL of methanol by heating on a water bath: the solution is transparent and clear.

(2) *Related substances:* Proceed as directed in the Purity (6) under Carbamazepine; no spot other than the principal spot with the R_f value of about 0.9 appears.

Nitrogen: 6.8% to 7.3% (Nitrogen Determination).

Imipramine hydrochloride $C_{19}H_{24}N_2 \cdot HCl$ [See monograph, Part I]

Indigo carmine $C_{16}H_8N_2Na_2O_6S_2$ [Special Class]

Indigo carmine TS Dissolve 0.22 g of indigo carmine in water to make 100 mL. Use within 60 days after preparation.

2,3-Indolinedione $C_8H_5NO_2$ [Special Class]

Indometacin $C_2H_{16}N_2$ [See monograph, Part I]

Iodine I [Special Class]

Iodine monobromide See iodine(II) bromide.

Iodine solution, 0.005 mol/L 1.269 g of Iodine (I : 126.90) is contained in 1000 mL.

Preparation: Before to use, pipet 10 mL of 0.05 mol/L iodine solution and add 1 mL of potassium iodine solution (2 in 5) and water to make exactly 100 mL.

Iodine trichloride ICl_3 [Special class].

Iodine trichloride TS Place 7.9 g of iodine trichloride and 8.7 g of iodine in separate flasks, dissolve with acetic acid (100), mix the two solutions, and add acetic acid (100) again to make 1000 mL.

Iodine TS Dissolve 14 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid and water to make 1000 mL. Store away from light (0.1 mol/L).

Iodine TS Mix 1 mL of 1 mol/L hydrochloric acid and 1 mL of 0.2% iodine-2% potassium iodide solution to make 100 mL (diastase and protease 500).

Iodine TS, 0.0002 mol/L Pipet 1 mL of 1 mol/L iodine TS, add water to make exactly 500 mL, pipet 10 mL of the solution, and add water to make exactly 100 mL. Prepare before use.

Iodine TS, 0.0004 mol/L Pipet 1 mL of 1 mol/L iodine TS, add water to make exactly 250 mL, pipet 10 mL of the solution, and add water to make exactly 100 mL. Prepare before use.

Iodine TS, 0.003 mol/L Dissolve 0.81 g of iodine and 1.44 g of potassium iodine in water to make 1 L (povidone, povidone ophthalmic solution).

Iodine TS, 1 mol/L Add 10 mL of water to 12.7 g of iodine and 25 g of potassium iodide, shake well to mix, and add water to make 100 mL.

Iodine TS, dilute To 1 volume of iodine TS, add 4 volumes of water.

Iodine-starch TS To 100 mL of starch TS, add 3 mL of dilute iodine TS.

Iodine(II) bromide IBr Blackish brown crystals or masses. Dissolves in water, ethanol (95), ether, carbon dioxide or acetic acid (100). Preserve in light-resistant glass containers in a dark place.

Melting point: About 40 ℃

Iodoethane C_2H_5I [Special Class]

Iodomethane CH_3I [Special Class]

Ion exchange resin column Pack ion exchange resin Dowex 50w × 2 in a chromatography column with the internal diameter 12 cm to make the height at about 10 cm. Wash it with 100 mL of distilled water and then thoroughly rinse with 50 mL of 5 mol/L hydrochloric acid. Finish washing when the washings become neutral. Then, wash it sufficiently with 70 mL of a mixture of methanol and 25% ammonia water (9 : 1). Again, wash it with water until the washings become neutral. Then, activate it with 50 mL of 6 mol/L hydrochloric acid. Wash the resulting solution with water until the solution becomes neutral. Then, wash it with 40% ethanol.

Ion exchange resin column Place 2 g of ion exchange resin Dowex 50w × 2, 50 to 100 mesh in a column, wash it with 50 mL of 1 mol/L sodium hydroxide TS, 100 mL of water, and 50 mL of 1 mol/L hydrochloric acid, and then rinse it with water until the Litmus paper becomes neutral (phenylephrine hydrochloride).

Ion exchange resin column, strong base Add water to strongly basic ion exchange resin Dowex 1 × 4 (Type CI, 100 to 200 mesh) for washing by decanting, and transfer into a glass column for chromatography (internal diameter 10 mm) with water until the layer length is about 3 cm, pass the column with 25 mL each of dilute hydrochloric acid twice, and wash the column with water until the passing solution becomes neutral and use the column with the layer length at 2 cm.

Ion exchange resin column, weak acid Weigh 10 g of mildly acidic ion exchange resin CG 50 (100 to 200 mesh), wash several times with 50 mL each of water, and transfer into a column for chromatography (internal diameter 10 mm) with water until the layer length is about 1 cm, pass the column with 50 mL of dilute hydrochloric acid, and wash the column with water until the eluted solution becomes neutral. Then, pass the column with 50 mL of ammonium chloride solution (1 in 20), wash with 50 mL of water, remove the excess resin with a pipet to make the layer length at 7 cm.

Ion exchange resin, strong acid Strongly acidic ion exchange resin prepared in good quality for column chromatography.

Iron Fe Forms of strips, sheets, granules or wires. Fe NLT 97.7%. It is attracted by a magnet.

Iron (III) chloride TS, 5% Dissolve 5 g of iron (III) chloride hexahydrate in water to make 100 mL.

Iron (III)·chloride-potassium hexacyanoferrate (III) TS Mix 100 mL of 1% iron (III)·chloride-1% acetic acid solution, 100 mL of 1% potassium hexacyanoferrate (III) solution and 75 mL of methanol. Prepare before use.

Iron chloride-methanol TS See Iron(III) chloride-methanol TS.

Iron powder Fe [Special Class]

Iron salicylate TS Dissolve 0.1 g of ammonium iron(III) sulfate in 50 mL of diluted sulfuric acid (1 in 250), and add water to make 100 mL. Take 20 mL of this solution, add 10 mL of a solution prepared by dissolving 0.23 g of sodium salicylate in water to make 20 mL, 4 mL of dilute acetic acid, 16 mL of sodium acetate TS and water to make 100 mL. Prepare before use.

Iron sulfide See iron(II) sulfide.

Iron-phenol TS Dissolve 1.054 g of ammonium iron(II) sulfate hexahydrate in 20 mL of water, add 1 mL of sulfuric acid and 1 mL of hydrogen peroxide (30), heat until effervescence ceases, and dilute with water to make 50 mL. To 3 volumes of this solution transferred to a volumetric flask, add sulfuric acid while cooling, to make 100 volumes, preparing the iron-sulfuric acid solution. Separately, distill the phenol again, discarding the first 10% and the last 5% of the volume, and collect the distillate, away from moisture, in a dry, tared, glass-stoppered flask of about twice the volume. Stopper the flask, cool with ice, breaking the top crust with a glass rod to ensure complete crystallization, and after drying, weigh the flask. To the glass-stoppered flask, add iron-sulfuric acid solution in 1.13 times the mass of phenol, stopper the flask, and allow to stand without cooling but with occasional shaking for dissolution of phenol. Then, shake the mixture vigorously and allow to stand in a dark place for 16 to 24 hours. To the mixture, add diluted sulfuric acid (10 in 21) equivalent to 23.5% of the mixture, mix well, transfer to a dry glass-stoppered bottle, and preserve in a dark place, protected from moisture. Use this solution within 6 months.

Iron-phenol TS, dilute Add 4.5 mL of water to 10 mL of iron-phenol TS. Prepare before use.

Iron(II) sulfate heptahydrate $FeSO_4 \cdot 7H_2O$ [Special class]

Iron(II) sulfate TS Dissolve 8 g of iron(II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare before use.

Iron(II) sulfide FeS [for hydrogen sulfide development].

Iron(II) tartrate TS Dissolve 1 g of iron(II) sulfate heptahydrate, 2 g of potassium sodium tartrate tetrahydrate and 100 mg of sodium bisulfite in water to make 100 mL.

Iron(II) thiocyanate TS Add 3 mL of dilute sulfuric acid to 35 mL of water and boil to remove dissolved oxygen. To this hot solution, add 1 g of iron(II) sulfate hexahydrate to dissolve, cool, and then add 0.5 g of potassium thiocyanate to dissolve. The solution exhibits a pale red color; add reduced iron to decolorize, decant, remove excess reduced iron, and then store while keeping away from oxygen. The solution exhibiting a pale red color is not used.

Iron(II) trisodium pentacyanoamine TS To 1.0 g of sodium pentacyanonitrosylferrate(III) dihydrate, add 3.2 mL of ammonia TS, shake to mix, and allow to stand with the stopper closed overnight. Add this solution in 10 mL of ethanol (99.5), collect the produced yellow precipitates by filtering under suction, wash the precipitates with anhydrous ether, dry, and store in the desiccator. Dissolve in water to make an 1.0 mg/mL solution, and store in a refrigerator before use. Use within 7 days after dispensing.

Iron(III) chloride hexahydrate $FeCl_3 \cdot 6H_2O$ [Special Class]

Iron(III) chloride TS Dissolve 9 g of Iron(III) chloride hexahydrate in water to make 100 mL (0.33 mol/L).

Iron(III) chloride TS, acidic To 60 mL of acetic acid (100), add 5 mL of sulfuric acid and 1 mL of Iron(III) chloride TS.

Iron(III) chloride TS, dilute Add water to 2 mL of dilute Iron(III) chloride TS to make 100 mL. Prepare before use.

Iron(III) chloride-acetic acid TS Dissolve 0.1 g of Iron(III) chloride hexahydrate in diluted acetic acid (31) (3 in 100) to make 100 mL·

Iron(III) chloride-iodine TS Dissolve 5 g of Iron(III) chloride hexahydrate and 2 g of iodine with a mixture of 50 mL of acetone and 50 mL of a solution of tartaric acid (1 in 5).

Iron(III) chloride-methanol TS Dissolve 1 g of Iron(III) chloride hexahydrate in methanol to make 100 mL.

Iron(III) chloride-potassium hexacyanoferrate(III) TS Dissolve 0.1 g of potassium hexacyanoferrate(III) in 20 mL of Iron(III) chloride TS. Prepare before use.

Iron(III) chloride-pyridine TS, anhydrous Weigh 1.7 g of anhydrous Iron(III) chloride hexahydrate and heat slowly by direct application of flame for dissolution and solidify it. Cool it, dissolve it in 100 mL of chloroform, add 8 mL of pyridine to mix, and filter.

Iron(III) nitrate nonahydrate $Fe(NO_3)_3 \cdot 9H_2O$ [Special Class]

Iron(III) nitrate TS Dissolve 1 g of Iron(III) nitrate in pH 2.0 hydrochloric acid-potassium chloride buffer solution to make 300 mL.

Iron(III) perchlorate See iron(III) perchlorate hexahydrate.

Iron(III) perchlorate hexahydrate $Fe(ClO_4) \cdot 6H_2O$ Hygroscopic, pale purple crystal. Its solution in ethanol (99.5) (1 in 125) is transparent and clear reddish orange.

Iron(III) perchlorate-ethanol (99.5) TS Dissolve 0.8 g of iron(III) perchlorate hexahydrate in perchloric acid-ethanol (99.5) TS to make 100 mL. Preserve in tight containers in a cold place.

Iron(III) sulfate *n* hydrate $Fe_2(SO_4)_3 \cdot nH_2O$ [Special class]

Iron(III) sulfate TS Dissolve 50 g of iron(III) sulfate *n*-hydrate in an excess of water, and add 200 mL of sulfuric acid and water to make 1000 mL.

Isatin See 2,3-indolinedione.

Isatin solution Dissolve 0.4 g of isatin 0.4 g in concentrated sulfuric acid to make 100 mL (doxazosin mesilate).

Isoamyl acetate $CH_3COOCH_2CH_2CH(CH_3)_2$ A clear and colorless liquid.

Boiling point: About 140 ℃

Specific gravity d_{20}^{20}: about 0.868 to 0.879

Refractive index n_D^{20}: about 1.395

Packaging and storage: Preserve in light resistant, tight containers.

Isoamyl acetate solution for penicillin See Isoamyl acetate solution, for penicillin.

Isoamyl alcohol See 3-methyl-1-butanol.

Isoamyl p-Hydroxybenzoate $C_{12}H_{16}O_3$ A white crystalline powder, having a slightly characteristic odor. It is very soluble in acetonitrile, ethanol (95), acetone or ether, and practically insoluble in water.

Melting point: 62 to 64 ℃

Isobutanol See 2-methyl-1-propanol.

Isobutyl p-hydroxybenzoate $C_{11}H_{14}O_3$ White crystals or crystalline powder without odor. Freely soluble in ethanol, acetone or ether, and practically insoluble in water.

Melting point: 75 to 77℃

Residue on ignition: NMT : 0.1%.

Content: NLT 99.0%

Assay: Perform the test as directed under the Assay of Ethyl Paraoxybenzoate in the monograph, Part II.

Each mL of 1 mol/L sodium hydroxide VS
= 194.23 mg of $C_{11}H_{14}O_3$

Isobutyl salicylate $C_{11}H_{14}O_2$ Colorless, clear liquid.

Refractive index n_D^{20}: 1.506 to 1.511

Boiling point: 260℃ to 262 ℃

Specific gravity d_4^{20}: 1.068 to 1.073

Purity: Perform the test with 1 μL of isobutyl salicylate as

directed under the Gas Chromatography according to the following conditions. Measure each peak area by the automatic integration method, and determine the amount of isobutyl salicylate by the percentage peak area method; NLT 97.0%.

Operating conditions

Detector: Thermal conductivity detector

Column: A column about 3 mm in the internal diameter and about 2 m in the length, packed with diatomaceous earth for gas chromatography (180 μm to 250 μm in particle diameter) that is coated with polyethylene glycol 20 M for gas chromatography in the ratio of 10%.

Carrier gas: Helium

Flow rate: About 20 mL per minute.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of isobutyl salicylate obtained from 1 μL of isobutyl salicylate composes about 60% to 80% of the full scale.

Time span of measurement: About 3 times the retention time of isobutyl salicylate.

Isoniazid $C_6H_7N_3O$ [See monograph]

Isoniazid TS Dissolve 0.1 g of isoniazid for quantification in 50 mL of methanol and 0.12 mL of hydrochloric acid, and add methanol to make 200 mL.

Isoniazid TS Dissolve 50 mg of isoniazid in ethanol to make 100 mL and add 0.53 mL of hydrochloric acid (prednisolone).

Isoniazid TS (for norethisterone assay) Dissolve 1.0 g of isoniazid for assay in 1000 mL of anhydrous methanol and add 1.3 mL of hydrochloric acid for mixing.

Isoniazid TS, 0.02 mol/L Dissolve 2.744 g of isoniazid in about 800 mL of methanol by shaking and add 3.6 mL of perchloric acid and methanol to make 1 L. Preserve in a brown bottle (cyproterone acetate).

Isoniazid, for assay $C_6H_7N_3O$ [See monograph, Part I. When dried, it contains NLT 99.0% of isoniazid ($C_6H_7N_3O$)]

Isonicotinic acid $C_6H_6NO_2$ White crystals or powder.

Melting point: About 315 ℃ (with decomposition)

Isonicotinic acid hydrazide See Isoniazid.

Isooctane See octane, iso.

Isopropanol See 2-propanol.

Isopropanol for liquid chromatography See 2-propanol for liquid chromatography.

Isopropyl 4-aminobenzoate $H_2NC_6H_4COOCH(CH_3)_2$ Pale brown crystals.

Melting point: 83 to 86 ℃

Isopropyl benzoate $C_6H_5COOCH(CH_3)_2$ A clear, colorless liquid, having a characteristic odor.

Refractive index n_D^{20}: 1.490 to 1.498

Specific gravity d_{20}^{20}: 1.007 to 1.016

Isopropyl ether See propyl ether, iso.

Isopropyl myristate $C_{17}H_{34}O_2$ Colorless, clear, oily liquid. It is odorless. Coagulate at about 5 ℃. Soluble in 90% alcohol, miscible with many organic solvents or solid oils, insoluble in water, glycerin or propylene glycol.

Refractive index n_D^{20}: 1.432 to 1.436

Specific gravity d_{20}^{20}: 0.846 to 0.854

Saponification value: 202 to 212

Acid value: NMT 1.

Iodine value: NMT 1.

Residue on ignition: NMT 0.1% (1 g).

Isopropyl myristate for sterility test See Isopropyl myristate, for sterility test.

Isopropyl myristate, for sterility test $C_{17}H_{34}O_2$ Transfer 100 mL of isopropyl myristate into a centrifuge tube, add 100 mL of twice-distilled water, and shake vigorously to mix for 10 minutes. Then centrifuge at a rate of 1800 revolutions per minute for 20 minutes, take the clear supernatant (isopropyl myristate layer) separately. If the pH of the residual water layer is less than 5.5, proceed with the clear supernatant as follows. Pass 500 mL of isopropyl myristate, which has met the requirements of pH measurement, through a glass column of 20 mm × 20 cm filled with activated alumina up to a 15 cm height. At this time, pass with a slightly positive pressure in order to facilitate adequate flow, and then sterilize isopropyl myristate again by filtration.

Isopropyl *p*-aminobenzoate See isopropyl 4-aminobenzoate.

Isopropyl *p*-Hydroxybenzoate $C_{10}H_{12}O_3$ White crystals or crystalline powder without odor. Isopropyl p-Hydroxybenzoate is freely soluble in ethanol (95), acetone and ether, practically insoluble in water.

Melting point: 84 to 86℃

Residue on ignition: NMT : 0.1%.

Content: NLT 99.0%

Assay: Perform the test as directed under the Assay of Ethyl Paraoxybenzoate in the monograph, Part II.

Each mL of 1 mol/L sodium hydroxide VS
= 180.20 mg of $C_{10}H_{12}O_3$

***N*-Isopropyl-4-iodoamphetamine hydrochloride** See N-isopropyl-4-iodoamphetamine hydrochloride.

***N*-isopropyl-4-iodoamphetamine hydrochloride** $C_{12}H_{18}IN \cdot HCl$ Colorless crystals or white powder. Freely soluble in water or methanol and slightly soluble in ether.

Melting point: 160 ℃ to 165 ℃

Isopropylamine See propylamine, iso.

Isopropylamine-ethanol TS To 20 mL of isopropylamine, add ethanol (99.5) to make 100 mL. Prepare before use.

Isopropylantipyrine See propylamine, iso.

Isotonic sodium chloride injection [See monograph, Part II]

Kainic acid $C_{10}H_{15}NO_4 \cdot H_2O$ [See monograph, Part I on Kainic Acid Hydrate]

Kanamycin sulfate $C_{18}H_{36}N_4O_{11} \cdot xH_2SO_4$ [See monograph, Part I]

Karl Fischer anolyte A Dissolve 100 g of diethanolamine in

900 mL of a mixture of methanol for Karl Fischer titration and chloroform for Karl Fischer titration (1 : 1). Pass dried sulfur dioxide gas through the solution while cooling. When the mass increase of the solution reaches 64 g, dissolve 20 g of iodine in the solution, and add water dropwise until the color of the solution changes from brown to yellow. Add 400 mL of chloroform for Karl Fischer titration to 600 mL of this solution.

Karl Fischer TS See the Water Determination under the General Tests.

Kerosin Usually, a methane hydrocarbon mixture. Clear, colorless liquid. It has a non-repellent, characteristic odor.

Specific gravity: About 0.80

Melting point:180 to 300 ℃

Kininogen Kininogen obtained by purification from bovine plasma. Perform the test according to the following procedure with the test solution, prepared to contain 1 mg of kininogen in 10 mL by dissolving a suitable volume of Kininogen in 0.02 mol/L phosphate buffer solution (pH 8.0); the following criteria are met.

(1) Add 0.1 mL of trichloroacetic acid solution (1 in 5) to 0.5 mL of the freshly prepared test solution, shake it for mixing, and centrifuge. To 0.5 mL of the clear supernatant, add pH 0.5 mL of 8.0 gelatin-tris buffer solution, and mix by shaking. To 0.1 mL of this solution, add 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution. Perform the test with 0.1 mL of this solution as directed in Purity (2) under Kallidinogenase to determine the amount of kinin: no kinin is detected.

(2) Warm 0.5 mL of the test solution at 30 ± 0.5 ℃ for 20 minutes and proceed as directed in (1): no kinin is detected.

(3) Perform the test with 0.5 mL of the test solution as directed in Purity (2) under Kallidinogenase: no decomposition of bradykinin is recognized.

(4) Add 0.5 mL of pH 8.0 0.02 mol/L phosphate buffer solution containing 500 g of crystallized trypsin previously warmed for 5 minutes at 30 ± 0.5 ℃ to 0.5 mL of the test solution, warm it at 30 ± 0.5 ℃ for 5 minutes, and add 0.2 mL of trichloroacetic acid solution (1 in 5), and shake well for mixing. Boil it for 3 minutes, immediately cool the mixture with ice, and centrifuge it. To 0.5 mL of the clear supernatant, add 0.5 mL of pH 8.0 gelatin-tris buffer solution, and mix by shaking. To 0.1 mL of this solution, add 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution, add trichloroacetic acid-gelatin-tris buffer solution to make 20 mL, and proceed as directed in (1) to determine the amount of kinin B_K per 1 well. Obtain the releasability of kinin in 1 mg of Kininogen using the following equation: the kinin releasability as bradykinin is NLT 10 μg/mg.
Kinin releasability in 1 mg of Kininogen

$$\text{(Equivalent to 1 μg of bradykinin/mg)} = B_K \times 0.96$$

Kininogen TS Dissolve a suitable amount of kininogen in pH 8.0 0.02 mol/L phosphate buffer solution to prepare a solution with the kinin releasability of NLT 1 μg of bradykinin in 1 mL of this solution.

Lactate buffer solution, 0.1 mol/L See lactate buffer solution, 0.1 mol/L.

Lactate buffer solution, 0.1 mol/L, pH 3.0 See lactate buffer solution, 0.1 mol/L, pH 3.0.

Lactate solution, 0.1 mol/L See lactate solution, 0.1 mol/L.

Lactic acid $CH_3CH(OH)COOH$ [Special class]

Lactic acid TS Dissolve 12.0 g of lactic acid in water to make 100 mL.

Lactic acid TS See lactic acid TS.

Lactobionic acid $C_{12}H_{22}O_{12}$ Colorless crystals or pale white crystalline powder.

Melting point: 113 ℃ to 118 ℃

Purity: Weigh about 0.1 g of lactobionic acid, dissolve in 10 mL of a mixture of methanol and water (3 : 2), and use this solution as the test solution. With this solution, perform the test as directed under the Thin Layer Chromatography. Spot 10 μL of the test solution onto a thin-layer plate made of silica gel for thin-layer chromatography, develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (3 : 3 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105 ℃ for 20 minutes; any spot other than the principal spot does not appear.

Lactose See lactose monohydrate.

Lactose broth Add lactose monohydrate to normal broth at a ratio of 0.5%, and then add about 12 mL of bromothymol blue-sodium hydroxide TS to 1000 mL of the medium. Then, dispense about 10 mL each of the solution into tubes for fermentation, and sterilize fractionally once daily at 100°C for 15 to 30 minutes on three successive days by using a steam sterilization pot or sterilize by autoclaving for not longer than 20 minutes at 121°C and cool immediately by immersing in cold water.

Lactose broth, 2x concentrated Add lactose monohydrate to normal broth prepared by using 500 mL instead of 1000 mL of water, at a ratio of 1.0%, and prepare as directed under Lactose Broth.

Lactose broth, 3x concentrated Add lactose monohydrate to normal broth prepared by using 330 mL instead of 1000 mL of water, at a ratio of 1.5%, and prepare as directed under Lactose Broth. Dispense 2.5 mL each into tubes for fermentation.

2x Concentrated lactose broth See lactose broth, 2x concentrated.

3x Concentrated lactose broth See lactose broth, 3x.

Lactose monohydrate $C_{12}H_{22}O_{11}\cdot H_2O$ [See monograph, Part II on Lactose]

Lactose substrate TS Dissolve 6.0 g of saccharides in pH 4.5 sodium monohydrogen phosphate-citric acid buffer solution to make 100 mL.

***α*-Lactose-β-lactose mixture (1 : 1)** Use a mixture of lactose and anhydrous lactose (3 : 5).

Lanthanum chloride hexahydrate $BaCl_2\cdot 2H_2O$ [Special Class]

Lanthanum chloride solution Dissolve 26.7 g of lanthanum chloride heptahydrate in 0.125 mol/L hydrochloric acid to make 100 mL.

Lanthanum chloride TS Slowly add 10 mL of hydrochloric acid to lanthanum(III) oxide 5.9 g and heat it. After cooling, add water to make 100 mL.

Lanthanum nitrate $La(NO_3)_3 \cdot 6H_2O$ [Special Class]

Lanthanum oxide TS Dissolve 2g of lanthanum oxide (La_2O_3) in 20 mL of hydrochloric acid, and add water to make 100 mL (calcium).

Lanthanum oxide La_2O_3 [Special class].

Lanthanum(III) oxide La_2O_3 A white crystal.
Loss on drying: NMT 0.5% (1 g, 1000 ℃, 1 hours)

Lauromacrogol [See monograph]

Lead acetate See lead acetate trihydrate.

Lead acetate paper Usually, immerse strips of filter paper, 6 cm to 8 cm in size, in lead acetate TS, drain off the excess liquid, and dry the paper at 100 ℃, avoiding contact with metals.

Lead acetate trihydrate $Pb(CH_3COO)_2 \cdot 3H_2O$ [Special class]

Lead acetate TS Add freshly boiled and cooled water to 9.5 g of lead acetate trihydrate, and dissolve to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/L).

Lead acetate TS Dissolve 1.5 g of lead acetate in 20 mL of water, and add 5 drops of acetic acid (sodium molybdate).

Lead acetate TS See lead acetate TS.

Lead dioxide See lead(IV) oxide.

Lead monoxide See lead(II) oxide.

Lead nitrate See lead(II) nitrate.

Lead subacetate TS Grind 3 g of lead(II) acetate and 1 g of lead(II) oxide with 05 mL of water for mixing, place the sulfur-colored mixture obtained therefrom into a beaker, cover with a watch glass and heat on a water bath. When the resulting solution exhibits uniformly white or reddish white color, add 9.5 mL of hot water in small volumes, cover with a watch glass again and allow to stand. Then, take the clear supernatant by decanting, and add water to adjust the specific gravity to 1.23 to 1.24 (15 ℃). Store in a stoppered container.

Lead subacetate TS, dilute Add freshly boiled and cooled water to 2 mL of lead subacetate TS to make 100 mL. Prepare before use.

Lead(II) nitrate $Pb(NO_3)_2$ [Special Class]

Lead(II) oxide PbO Dark brown to blackish brown, powder or grains.

Identification: The clear supernatant of a solution of lead(II) oxide in dilute acetic acid (1 in 100) responds to Qualitative Analysis (3) for lead salt.

Lead(IV) oxide PbO_2 [Special class].

***L*-Leucine** $C_6H_{13}NO_2$ [See monograph]

Levothyroxine sodium $C_{15}H_{11}I_4NaO_4 \cdot nH_2O$ [See monograph, Part I]

Lime milk (Calcium hydroxide) Take 10 g of calcium oxide in a mortar, and triturate while mixing on the slow addition of 40 mL of water.

Limonene $C_{10}H_{16}$ Clear and colorless liquid, having a specific aroma and a slightly taste.

Refractive index n_D^{20}: 1.472 to 1.474
Specific gravity d_{20}^{20}: 0.841 to 0.846
Melting point: 176 ℃ to 177 ℃
Purity *Related substances*: Dissolve 0.1 g of limonene in 25 mL of hexane and use this solution as the test solution. Perform the test with 2 μL of this solution as directed under the Gas Chromatography according to the following conditions. Measure each peak area by the automatic integration method and determine the amount of limonene by the percentage peak area method; it is NLT 97.0%.

Operating conditions

Follow the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of area measurement.

Detection sensitivity: To 1 mL of the test solution, add hexane to make 100 mL. Adjust the detection sensitivity so that the peak height of limonene obtained from 2 mL of this solution is 40% to 60% of the full scale.

Time span of measurement: About 3 times the retention time of limonene after the solvent peak.

Liothyronine sodium $C_{15}H_{11}I_3NNaO_4$ [See monograph, Part I]

Liquid paraffin See paraffin, liquid.

Lithium acetate dihydrate $CH_3COOLi \cdot 2H_2O$ Colorless crystals.

Lithium chloride LiCl [Special Class]

Lithium perchlorate $LiClO_4 \cdot HCl$ [Special class]

Lithium sulfate See lithium sulfate monohydrate.

Lithium sulfate monohydrate $Li_2SO_4 \cdot H_2O$ [Special class]

Litmus milk Allow the fresh whole milk to stand in a refrigerator for a day and a half, and siphon the skim milk carefully avoiding the cream layer. Heat with steam for 1 hour, cool in a refrigerator, filter, weigh the filtrate, and add a sufficient amount of litmus solution until it exhibits a bluish purple color. Sterilize at 115 ℃ for 10 minutes. The medium becomes colorless when heated and returns to its original color when cooled (*Streptococcus faecalis*).

Litmus paper, blue [Blue litmus paper].

Litmus paper, red [Red litmus paper].

Locke-Ringer's TS

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride hydrate	0.24 g

Magnesium chloride hexahydrate	0.2 g
Sodium bicarbonate	0.5 g
Glucose	0.5 g
Water, freshly distilled with a hard-glass flask	A sufficient quantity
Total volume	1000 mL

Prepare before use. The componets except glucose and sodium hydrogen carbonate may be made with concentrated crude solutions, stored in a cold place, and diluted before use.

Loganin for assay See loganin, for assay.

Loganin, for assay $C_{17}H_{26}O_{10}$ White crystals or crystalline powder. Soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol.

Melting point: 221 ℃ to 227 ℃

Absorbance: $E_{1cm}^{1\%}$ (235 nm): 275 to 303 (dried in a silica gel desiccator for 24 hours, 5 mg, methanol, 500 mL).

Purity *Related substances*: Dissolve 2 mg of loganin for assay in 5 mL of the mobile phase, and use this solution as the test solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of each solution by the automatic integration method; the total area of the peaks other than the peak of loganin from the test solution is not larger than the peak area of loganin from the standard solution.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 238 nm)

Column: A stainless steel column 4.6 mm in internal diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μL in particle diameter).

Column temperature: Constant temperature around 80 ℃

Mobile phase: A mixture of water, acetonitrile and methanol (55 : 4 : 1)

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes)

System suitability

System performance: Proceed with 10 μL of the standard solution according to the above operating conditions; the number of theoretical plates and the symmetry factor of the peak of loganin are NLT 5000 and NMT 1.5, respectively.

System repeatability: Repeat the test 6 times with 10 μL each of the standard solution according to the above conditions; the relative standard deviation of the peak areas of loganin is NMT 1.5%.

Lysate reagent A lyophilized product prepared from blood cell extracts of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). Prepared by removing the factor G reacting to β-glucans or by inhibiting the factor G reacting system.

Lysate TS Dissolve lysate reagent in water for bacterial endotoxins test, or in a suitable buffer solution, by gentle stirring.

***L*-Lysine hydrochloride** $C_6H_{14}NO_2{\cdot}HCl$ [See monograph, Part I]

Magaesium sulfate TS Dissolve 12 g of magnesium sulfate heptahydrate in water to make 100 mL (0.5 mol/L).

Magnesia TS Dissolve 5.5 g of magnesium chloride and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, transfer to a stoppered bottle, allow to stand for few days, and filter. If the solution is not clear, filter before use.

Magnesium Mg [Special class]

Magnesium buffer solution pH 9.0 To 3.1 g of boric acid and 500 mL of water, add 21 mL of 1 mol/L sodium hydroxide solution and 10 mL of 0.1 mol/L magnesium chloride solution, and add water to make 1000 mL

Magnesium chloride See magnesium chloride hexahydrate.

Magnesium chloride hexahydrate $MgCl_2{\cdot}6H_2O$ [Special Class]

Magnesium nitrate hexahydrate $Mg(NO_3)_2{\cdot}6H_2O$ [Special Class]

Magnesium oxide MgO [Special class].

Magnesium powder Mg [Special class]

Magnesium sulfate See magnesium sulfate heptahydrate.

Magnesium sulfate heptahydrate $MgSO_4{\cdot}7H_2O$ [Special class]

Magneson $C_{12}H_9N_3O_4$ [Special class]

Magneson indicator Dissolve 0.2 g of magneson in benzene to make 100 mL (hymecromone).

Malachite green See malachite green oxalate.

Malachite green oxalate $C_{52}H_{54}N_4O_{12}$ [Malachite (oxalate), Special class].

Malachite green TS Dissolve 1 g of malachite green oxalate in 100 mL of acetic acid (100).

Maleic acid $C_4H_4O_4$ [Special class] White crystalline powder.

Identification: Determine the infrared spectrum of maleic acid as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1706 cm^{-1}, 1637 cm^{-1}, 1587 cm^{-1}, 1567 cm^{-1}, 1436 cm^{-1}, 1263 cm^{-1}, 876 cm^{-1} and 786 cm^{-1}.

Maltose $C_{12}H_{22}O_{11}{\cdot}H_2O$ [Special class]

Manganese dioxide MnO_2 [Special Class]

Manganese sulfate See manganese sulfate hydrate

Manganese sulfate hydrate $MnSO_4{\cdot}H_2O$ [Special class]

Manganese sulfate solution Dissolve 10 g of manganese sulfate in 50 mL of water and mix with a mixture of 20 mL of sulfuric acid, 50 mL of water, and 20 mL of phosphoric acid (ferrous chloride).

***D*-Mannitol** $C_6H_{14}O_6$ [See monograph, D-Mannitol]

***D*-Mannosamine hydrochloride** $C_6H_{13}NO_5 \cdot HCl$ [Special class] (3S,4R,5S,6R)-3-amino-6-(hydroxymethyl)oxane-2,4,5-triol hydrochloride
White powder.
Melting point: About 168 ℃ (with decomposition)
Specific optical rotation $[a]_D^{20}$: -4.2° to -3.2° (0.4 g, water, 20 mL, 100 mm)

***D*(+)-Mannose** $C_6H_{12}O_6$ White crystal or crystalline powder. Very soluble in water.
Melting point: 132 ℃ (with decomposition)
Specific optical rotation $[a]_D^{20}$: + 13.7° to + 14.7° (4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm)

Mayer's TS Dissolve 1.358 g of mercury(II) chloride in 60 mL of water. Separately dissolve 5 g of potassium iodide in 10 mL of water. Mix these tow solutions well and add water to make 100 mL.

McIlvaine buffer solution, pH 5.0 Mix 10.3 mL of 0.1 mol/L disodium hydrogen phosphate TS and 9.7 mL of 0.1 mol/L anhydrous citric acid.

McIlvaine buffer solution, pH 5.6 Mix 3.4 mL of 0.1 mol/L anhydrous citric acid and 11.6 mL of 0.2 mol/L disodium hydrogen phosphate TS (diastase·protease 500).

McIlvaine buffer solution, pH 6.0 Mix a certain amount of 0.1 mol/L disodium hydrogen phosphate TS with 0.1 mol/L anhydrous citric acid to adjust the pH to 6.0.

Meat extract Beef extract or equivalent.

Meat peptone See meat peptone.

Meglumine $C_7H_{17}NO_5$ [See monograph, Part I]

Menthol $C_{10}H_{20}O$ [See monograph, Part II on *dl*-Menthol or *l*-Menthol].

Mercaptoacetic acid $HSCH_2COOH$ [Special class] Place in an ampoule, and preserve in a dark, cold place. Do not store for a long period.

2-Mercaptoethanol $C2H_6OS$ [Special class]

Mercaptopurine $C_5H_4N_4S \cdot H_2O$ [See monograph, Part I]

Mercuric acetate See mercury(II) acetate.

Mercuric acetate TS for non-aqueous titration See mercury(II) acetate TS, for non-aqueous titration.

Mercuric acetate TS for non-aqueous titration See mercury(II) acetate TS, for non-aqueous titration.

Mercuric acetate TS, for non-aqueous titration See mercury(II) acetate TS, for non-aqueous titration.

Mercuric ammonium thiocyanate TS Dissolve 30 g of ammonium thiocyanate and 27 g of mercury(II) chloride to make 1000 mL.

Mercuric bromide See mercury(II) bromide.

Mercuric bromide paper See mercury(II) bromide paper.

Mercuric chloride See mercury(II) chloride.

Mercuric chloride TS See mercury(II) chloride TS.

Mercuric nitrate TS See mercury(II) nitrate TS.

Mercuric oxide, yellow See mercury(II) oxide, yellow.

Mercuric sulfate TS See mercury(II) sulfate TS

Mercury Hg [Special class]

Mercury oxide yellow See mercury(II) oxide, yellow.

Mercury thiocyanate TS Dissolve 27 g of mercuric chloride and 30 g of ammonium thiocyanate in water to make 1000 mL (zinc oxide).

Mercury(II) acetate $Hg(CH3COO)_2$ [mercury(II) acetate, Special class]

Mercury(II) acetate TS for non-aqueous titration See mercury(II) acetate TS, for non-aqueous titration.

Mercury(II) acetate TS, for non-aqueous titration Dissolve acetic acid for non-aqueous titration in 5 g of mercury(II) acetate to make 100 mL.

Mercury(II) bromide $HgBr_2$ [Special class].

Mercury(II) bromide paper Cut a paper for chromatography into strips, about 4 cm in width and about 10 cm in length. Immerse these strips in a solution prepared by dissolving 5 g of mercury(II) bromide in 100 mL of ethanol (95), employing gentle heat to facilitate desolution, in a dark place for about 1 hour. Remove the paper from the solution without touching the portion of the strip which is used in the test, and allow it to dry spontaneously by suspending it from glass rods. After drying, cut off the circumference of the strip, making it about 20 mm^2 in area, and then cutoff every corner. Preserve in a dark place, protected from light.

Mercury(II) chloride $HgCl_2$ [Special Class]

Mercury(II) chloride TS Dissolve 6.5 g of mercury(II) chloride in water to make 100 mL.

Mercury(II) nitrate TS Dissolve 40 g of mercuric oxide yellow in a mixture of n32 mL of itric acid and 15 mL of water (4 mol/L). Preserve in a stoppered, light-resistant container.

Mercury(II) oxide yellow See mercury(II) oxide, yellow.

Mercury(II) oxide, yellow HgO [Special class] Store away from light.

Mercury(II) sulfate TS Mix 5 g of yellow mercury(II) oxide with 40 mL of water, and add slowly 20 mL of sulfuric acid while stirring, then, add another 40 mL of water, and stir until completely dissolved.

Metanil yellow $C_{18}H_{14}N_3NaO_3S$ Yellowish brown powder. Sparingly soluble in water, and very slightly soluble in ethanol

(95) or *N*,*N*-dimethylformamide.

Metanil yellow TS Dissolve 0.1 g of metanil yellow in 200 mL of *N*,*N*-dimethylformamide.

Metaphosphoric acid HPO_3 Colorless, deliquescent sticks or masses.

Identification (1) Dissolve 1 g of metaphosphoric acid in 50 mL of water, and use this solution as the test solution. To 10 mL of this solution, add 0.2 mL of ammonia TS and 1 mL of silver nitrate TS; a yellowish white precipitate is produced.

(2) To 10 mL of the test solution obtained in (1), add 10 mL of albumin TS; a white precipitate is produced.

Metaphosphoric acid-acetic acid TS Dissolve 15 g of metaphosphoric acid and 40 mL of acetic acid (100) in water to make 500 mL. Preserve in a cold place. Use within 2 days.

Methacrylate carboxylic acid cation exchange resin [First class]

Methanesulfonic acid CH_3SO_3H Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, ethanol (95) or ether.

Specific gravity d_{20}^{20}: 1.483 to 1.488

Congealing temperature: 15 ℃ to 20 ℃

Content: NLT 99.0%.

Assay: Weigh accurately about 2 g of methanesulfonic acid, dissolve in 40 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS
= 96.11 mg of CH_3SO_3H

Methanesulfonic acid TS To 35 mL of methanesulfonic acid, add 20 mL of acetic acid (100) and water to make 500 mL.

Methanesulfonic acid TS, 0.1 mol/L To 4.8 g of methanesulfonic acid, add water to make 500 mL.

Methanol CH_3OH [Special class]

Methanol for Karl Fischer titration See the Water Determination under the General Tests.

Methanol, anhydrous CH_4O To 1000 mL of methanol, add 5 g of magnesium powder. If necessary, add 0.1 mL of mercury(II) chloride TS to start the reaction. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate away from moisture. Water content is NMT 0.3 mg/mL.

Methanol, for water determination See the Water in the General Tests.

Methanol, purified Distill methanol before use.

Methanolic hydrochloric acid TS, 0.01 mol/L To 1 mL of 1 mol/L hydrochloric acid, add methanol to make 100 mL.

Methanolic hydrochloric acid TS, 0.1 mol/L To 10 mL of 1 mol/L hydrochloric acid, add methanol to make 100 mL.

Methanolic sulfuric acid, 0.05 mol/L To 5.0 g of 0.05 mol/L 98% sulfuric acid, add methanol to make 1 L (Use after allowing to stand for 24 hours) (bromazepam)

Methionine $C_5H_{11}NO_2S$ [See monograph, Part I on L-methionine].

2-Methooxyethanol for Karl Fischer titration See methyl cellosolve for Karl Fischer Titration in the Water Determination under the General Tests.

1-Methoxy-2-propanol $C_4H_{10}O_2$ Colorless, clear liquid.

Purity *Clarity of solution*: To 5 mL of 1-methoxy-2-propanol, add 20 mL of water, and shake to mix; the solution is clear.

Refractive index n_D^{20}: 1.402 to 1.405

Specific gravity d_{20}^{20}: 0.920 to 0.925

Water: NMT 0.5% (5 g).

Content: NLT 98% (Gas Chromatography). For the Assay, follow the corrected percentage peak area method.

Operating Conditions

Detector: Thermal conductivity detector

Column: A glass column about 3 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography (150 μm to 180 μm in particle diameter) that is coated with polyethylene glycol 20 M for gas chromatography in the ratio of 20%.

Column temperature: Constant temperature around 90 ℃

Carrier gas: Helium

Flow rate: 20 mL/min

2-Methoxy-4-methylphenol $C_8H_{10}O_2$ Colorless to pale yellow liquid. Miscible with methanol and ethanol, and slightly soluble in water. Congealing temperature: 3 ℃ to 8 ℃

Identification Determine the infrared absorption spectrum of 2-methoxy-4-methylphenol as directed in the ATR method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1511 cm-1, 1423 cm-1, 1361 cm-1, 1268 cm-1, 1231 cm-1, 1202 cm-1, 1148 cm-1, 1120 cm-1, 1031 cm-1, 919 cm-1, 807 cm-1 and 788 cm-1.

Purity *Related substances*: Perform the test with 0.2 μL of 2-methoxy-4-methylphenol as directed under the Gas Chromatography according to the following conditions. Determine each peak area by the automatic integration method; the total area of the peaks other than the peak of 2-methoxy-4-methylphenol is NMT 3.0%.

Operating conditions

Detector: Hydrogen ionization detector

Column: A fused silica column about 0.25 mm in internal diameter and 60 m in length, coated the inside surface with polymethylsiloxane for gas chromatography in 0.25 μm - 0.5 μm thickness.

Column temperature: Inject at a constant temperature of about 100 °C, raise the temperature to 130 °C at a rate of 5 °C per minute, raise to 140 °C at a rate of 2 °C per minute, raise to 200 °C at a rate of 15 °C per minute, and maintain at 200 °C for 2 minutes.

Melting point: About 200 ℃

Detector temperature: 250 ℃

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 2-methoxy-4-methylphenol is about 10 minutes.

Split ratio: 1 : 50

System suitability

System performance: Dissolve 60 mg of 2-methoxy-4-methylphenol in methanol to make 100 mL, and use this solution as the system suitability solution. Proceed with 1 μL of this solution under the above operating conditions; the symmetry factor

of the peak of 2-methoxy-4-methylphenol is NMT 1.5.

System repeatability: Repeat the test 6 times with 1 μL of the system suitability solution under the above operating conditions; the relative standard deviation of the peak area of 2-methoxy-4-methylphenol is NMT 2.0%.

4-Methoxybenzaldehyde-acetic acid TS To 0.5 mL of 4-methoxybenzaldehyde, add acetic acid (100) to make 100 mL.

4-Methoxybenzaldehyde-sulfuric acid TS To 9 mL of ethanol (95), add 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix thoroughly.

4-Methoxybenzaldehyde C8H8O2 Clear, colorless to pale yellow liquid. Miscible with ethanol (95) or ether, and practically insoluble in water.

Specific gravity d_{20}^{20}: 1.123 to 1.129

Content: NLT 97.0%.

Assay: Weigh accurately about 0.8 g of 4-methoxybenzaldehyde, add exactly 7.5 mL of hydroxylamine TS, shake well to mix, allow to stand for 30 minutes, and titrate with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS). However, the endpoint of titration is when the blue color of the solution turns to green and then finally to yellowish green. Perform a blank test in the same manner.

Each mL of 0.5 mol/L hydrochloric acid VS
= 68.08 mg of $C_8H_8O_2$

2-Methoxyethanol $CH_3OCH_2CH_2OH$ [Special class].

Methyl behenate $C_{23}H_{46}O_2$ White, scaly crystals or powder. It is odorless and tasteless. Dissolves in acetone, ether or chloroform.

Melting point: About 54 ℃

Saponification value: 155.5 to 158.5

Methyl benzoate $C_6H_5COOCH_3$ A clear, colorless liquid.

Refractive index n_D^{20}: 1.515 to 1.520

Specific gravity d_{20}^{20}: 1.087 to 1.095

Purity: Dissolve 0.1 mL of methyl benzoate in the mobile phase from the Assay under Thiamine Hydrochloride to make 50 mL. Perform the test with 10 μL of this solution as directed under the Liquid Chromatography according to the operating conditions in the Assay under Thiamine Hydrochloride, measure each peak area by the automatic integration method in a range about twice the retention time of the main peak, and determine the amount of methyl benzoate by the percentage peak area method; it is NLT 99.0%.

Methyl benzoate for estriol test See methyl benzoate, for estriol test.

Methyl benzoate, for estriol test $C_8H_8O_2$ A clear, colorless liquid, having a characteristic odor.

Refractive index n_D^{20}: 1.515 to 1.520

Specific gravity d_{20}^{20}: 1.087 to 1.095

Acid value: NMT 0.5.

Methyl butanoate $C_{23}H_{46}O_2$ White plate-like crystals or powder. It is odorless and tasteless. Dissolves in acetone, ether or chloroform.

Saponification value: 155.5 to 158.5.

Melting point: 54 ℃

Methyl cellosolve See 2-methoxyethanol.

Methyl ethyl ketone 2-butanone

Methyl ethyl ketone See 2-butanone.

Methyl iodide See iodomethane.

Methyl isobutyl ketone See 4-methyl-2-pentanone.

Methyl orange $C_{14}H_{14}N_3NaO_3S$ [Special class].

Methyl orange TS Dissolve 0.1 g of methyl orange in 100 mL of water. Filter, if necessary.

Methyl orange TS See methyl orange TS.

Methyl orange-xylene cyanol FF TS Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500 mL of dilute ethanol.

Methyl oxide-boric acid TS Dissolve 0.5 g of methyl orange and 5.2 g of boric acid in 500 mL of water by warming on a water bath. After cooling, wash this solution three times with 50 mL of chloroform.

Methyl p-Hydroxybenzoate $HOC_6H_4COOCH_3$ [See monograph, Part II]

Methyl red $C_{15}H_{15}N_3O_2$ [Special class]

Methyl red TS Dissolve 0.1 g of methyl red in 100 mL of ethanol. Filter, if necessary.

Methyl red TS See methyl red TS.

Methyl red TS for acid or alkali test See methyl red TS, for acid or alkali test.

Methyl red TS, dilute Dissolve 25 mg of methyl red in 100 mL of ethanol (99.5). Filter, if necessary. Prepare before use.

Methyl red TS, for acid or alkali test Dissolve 0.1 g of methyl red in 7.4 mL of 0.05 mol/L sodium hydroxide or 3.7 mL of 0.1 mol/L sodium hydroxide while triturating to mix in a mortar, and add freshly boiled and cooled water to make 200 mL.

Packaging and storage: Preserve in light-resistant, glass-stoppered bottles.

Methyl silicone polymer for gas chromatography Prepared for gas chromatography.

Methyl red-methylene blue TS Dissolve 0.1 g of methyl red and 0.1 g of methylene blue in ethanol (95) to make 100 mL. Filter, if necessary. Store away from light.

Methyl salicylate [See monograph, Part I]

Methyl yellow $C_{14}H_{15}N_3$ [Special class].

Methyl yellow TS Dissolve 0.1 g of methyl yellow in 200 mL of ethanol (95).

3-Methyl-1-butanol $C_5H_{12}O$ [Special class].

3-Methyl-1-phenyl-5-pyrazolone $C_{10}H_{10}N_2O$ [Special

class].

2-Methyl-1-propanol $(CH_3)_2CHCH_2OH$ [Special class].

4-Methyl-2-pentanone $CH_3COCH_2CH(CH_3)_2$ [Special class].

2-Methylamino pyridine for Karl Fischer titration See the Water Determination under the General Tests.

***p*-Methylaminobenzaldehyde-sulfuric acid TS** Dissolve 2.0 g of 4-dimethylaminobenzaldehyde in 8 mL of 80% sulfuric acid.

4-Methylaminopbenol sulfate TS Dissolve 0.35 g of 4-methylaminophenol sulfate and 20 g of sodium bisulfite in water to make 100 mL. Prepare before use.

***p*-Methylaminophenol sulfate TS** See 4-methylaminophenol sulfate TS.

4-Methylaminophenol sulfate $(HOC_6H_4NHCH_3)_2 \cdot H_2SO_4$ [Special class]

***p*-Methylaminophenol sulfate** See 4-methylaminophenol sulfate.

2-Methylaminopyridine $C_6H_8N_2$ Pale yellow liquid.

Boiling point: 200℃ to 202 ℃

Specific gravity d_{20}^{20}: 1.050 to 1.065

Water: NMT 1 mg per 1 g of 2-methylaminopyridine.

2-Methylaminopyrine See 2-methylaminopyridine.

***D*-(+)-α-Methylbenzylamine** $C_6H_5CH(CH_3)NH_2$ Colorless or pale yellow clear liquid, having an amine-like odor. Very soluble in ethanol (95) or acetone, and slightly soluble in water.

Refractive index n_D^{20}: 1.524 to 1.529

Specific gravity d_{20}^{20}: 0.948 to 0.956

Specific optical rotation $[a]_D^{20}$: +37° to +40° (50 mm).

Assay: Perform the test with 0.6 µL of D-(+)-α- methylbenzylamine as directed under the Gas Chromatography according to the following conditions. Determine each peak area by the automatic integration method. Determine the amount of D-(+)-α-methylbenzylamine by the percentage peak area method; it is NMT 98.0%.

Operating conditions

Detector: Flame ionization detector

Column: A glass column about 3 mm in internal diameter and about 2 m in length, packed with diatomaceous earth for gas chromatography (180 µm to 250 µm in particle diameter) coated with polyethylene glycol 20 M for gas chromatography and potassium hydroxide at the ratio of 10% and 5%, respectively.

Column temperature: Constant temperature around 140 ℃

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of D-(+)-α-methylbenzylamine is about 5 minutes.

Selection of column: To 5 mL of D-(+)-α-methylbenzylamine, add 1 mL of pyridine. Proceed with 0.6 µL of this solution under the above operating conditions; pyridine and D-(+)-α-methylbenzylamine are eluted in this order with the resolution between their peaks being less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of D(+)-α-methylbenzylamine is about 90% of the full scale.

Time span of measurement: About three times the retention time of D-(+)-α-methylbenzylamine.

Methyldopa $C_{10}H_{13}NO_4$ [See monograph, Part I]

Methylene blue $C_{16}H_{18}ClN_3S \cdot 3H_2O$ [Special class]

Methylene blue solution Dissolve 5 g of methylene blue in 100 mL of ethanol (99.5), and filter if necessary (Active lactobacillus sporogenes).

Methylene blue TS Dissolve 0.1 g of methylene blue in water to make 100 mL. Filter, if necessary.

***dl*-Methylephedrine hydrochloride** $C_{11}H_{17}NO \cdot HCl$ [See monograph, Part I]

Methylmethyloxaniline chloride TS See methylrosaniline chloride TS.

Methylne blue-sulfuric acid-sodium dihydrogen phosphate TS Dissolve 30 mL of methylene blue solution (1 in 1000) with 500 mL of water, 6.8 mL of sulfuric acid and 50 g of sodium dihydrogen phosphate dehydrate, and add water to make 1000 mL.

Methylprednisolone [See monograph, Part I]

Methylrosaniline chloride $C_{25}H_{30}ClN_3 \cdot 9H_2O$ [Special class]

Methylrosaniline chloride TS Dissolve 0.1 g of methylrosaniline chloride with 10 mL of acetic acid (100).

Methyltestosterone $C_{20}H_{30}O_2$ [See monograph, Part I]

Methylthymol blue $C_{37}H_{43}N_2NaO_{13}S$ [Special class]

Methylthymol blue sodium chloride indicator Mix 0.25 g of methylthymol blue and 10 g of sodium chloride, and triturate carefully until the mixture becomes homogeneous.

Methylthymol blue-potassium nitrate indicator Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate carefully until the mixture becomes homogeneous.

Sensitivity: Dissolve 20 mg of methylthymol blue-potassium nitrate indicator in 100 mL of 0.02 mol/L sodium hydroxide; the solution exhibits a slightly blue color. Add 0.05 mL of 0.01 mol/L barium chloride to this solution; the solution exhibits a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt;, the solution becomes colorless.

Metol TS Dissolve 1 g of 4-methylaminophenol sulfate and 20 g of dried sodium sulfite in water to make 100 mL (calcium phosphate choline chloride).

Milk casein See casein, milk.

Milk casein solution After determining loss on drying of milk casein (1g, 105 ℃, 4 hours), weigh accurately 1.2 g of dried milk casein, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, and heat to dissolve. After cooling, adjust the pH to 8.0 with 0.5 mol/L sodium hydroxide, and add water to make 200 mL

(semi-alkaline protease).

Milk casein TS, 1.5% Weigh accurately 1.5 g of milk casein, add 30 mL of 0.1 mol/L sodium hydroxide TS, heat it at 90 ℃ to 95 ℃ for 10 minutes to dissolve, cool, adjust the pH to 8.0 with dilute phosphoric acid (1 in 25), and then add 20 mL of pH 8.0 0.1 mol/L phosphate buffer solution and water to make 100 mL. It can be used for 45 days at temperatures not higher than 5 ℃ (Samprose).

Milk casein TS, 1.5%, pH 6.0 Weigh accurately 1.5 g of milk casein, calculated on the anhydrous basis, dissolve in 20 mL of 0.1 mol/L sodium hydroxide TS by warming, cool, adjust the pH to 6.0 with 0.1 mol/L phosphoric acid, and add 20 mL of phosphate buffer solution, pH 6.0, and water to make 100 mL. Prepare this test solution just before use.

Milk casein TS, 1.5%, pH 8.0 Weigh 1.5 g of milk casein, calculated on the anhydrous basis, dissolve in 30 mL of 0.1 mol/L sodium hydroxide TS by warming, cool, adjust the pH to 8.0 with 0.1 mol/L phosphoric acid, and add 20 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, and water to make 100 mL.

Millon TS Place 2 mL of mercury into a 200-mL Erlenmeyer flask, and add carefully 20 mL of nitric acid in a fume hood. As soon as the initial violent reaction subsides, stir if necessary. If there is a residual unreacted mercury, dissolve the residue with 35 mL of water and a sufficient amount of dilute nitric acid. Add 10% sodium hydroxide TS dropwise until the precipitates produced do not dissolve anymore. Add 5 mL of dilute nitric acid and mix well. Prepare this test solution just before use. This test solution is highly toxic.

Mixed peptone TS Dissolve 1.0 g of Neopeptone in 100 mL of 0.025 mol/L barbital TS. Use imidazole buffer solution instead of 0.025 mol/L barbital TS.

Molybdenum oxide-citric acid TS Dissolve 54 g of molybdenum(VI) oxide and 11 g of sodium hydroxide in 200 mL of water by heating while shaking to mix. Separately, dissolve 60 g of citric acid monohydrate in 250 mL of water, and add 140 mL of hydrochloric acid. Mix tow solutions, filter if necessary, add water to make 1000 mL, and add potassium bromate solution (1 in 100) until the solution exhibits a yellowish green color. Preserve in hermetic containers, protected from light.

Molybdenum trioxide See molybdenum(VI) oxide.

Molybdenum trioxide-citric acid TS See Molybdenum oxide-citric acid TS.

Molybdenum(VI) oxide White to yellowish green powder.

Identification: Dissolve 0.5 g of magnesium oxide in 5 mL of ammonia water (28). Take 1 mL of this solution, acidity with an appropriate amount of nitric acid, add 5 mL of sodium phosphate TS, and warm; yellow precipitates are formed.

Molybdovanadate TS Suspend 4 g of ammonium molybdate and 0.1 g of finely powdered ammonium vanadate in 70 mL of water, triturate until dissolved, add 20 mL of nitric acid and water to make 100 mL.

Monoethanolamine See 2-Aminoethanol.

Morpholine C_4H_9NO [Special class].

3-(*N*-Morpholino)propanesulfonic acid buffer solution, 0.02 mol/L, pH 7.0 Dissolve 4.2 g of 3-(*N*-Morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

3-(*N*-Morpholino)propanesulfonic acid buffer solution, 0.02 mol/L, pH 8.0 Dissolve 4.2 g of 3-(*N*-Morpholino)propanesulfonic acid in 700 mL of water, adjust the pH to 8.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

3-(*N*-Morpholino)propanesulfonic acid buffer solution, 0.1 mol/L, pH 7.0 Dissolve 20.92 g of 3-(*N*-Morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

3-(N-Morpholino)propanesulfonic acid buffer solution, 10 mmol/L, pH 7.0 Dissolve 2 g of sodium 3-(N-morpholino)propanesulfonate in 1000 mL of water, and adjust the pH to 7.0 with 5 mol/L sodium hydroxide.

Murexide $C_8H_8N_6O_6$ Reddish purple power. Practically insoluble in ethanol (95) or ether.

Purity *Clarity and color of solution*: Dissolve 10 mg of murexide in 100 mL of water; the solution is clear.

Residue on ignition: NMT 0.10% (1 g).

Sensitivity: Dissolve 10 mg of murexide in 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, add water to make 100 mL, and use this solution as the test solution. Separately, to 5 mL of diluted calcium standard solution (1 in 10), add 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, and water to make 25 mL, and adjust the pH to 11.3 with 0.1 mol/L sodium hydroxide TS. To this solution, add 2 mL of the test solution and water to make 50 mL; the solution exhibits a redish purple color.

Murexide-sodium chloride indicator Mix 0.1 g of murexide and 10 g of sodium chloride, and mix until the mixture becomes homogeneous. Store away from light.

Naphazoline nitrate $C_{14}H_{14}N_2 \cdot HNO_3$ [See monograph, Part I]

Naphthalene $C_{10}H_8$ [Special class].

Naphthalene-1,3-diolethanol TS, 0.2% Dissolve 0.1 g of naphthalene-1,3-diolethanol in ethanol to make 50 mL.

2-Naphthalenesulfonic acid hydrate $C_{10}H_8O_3S \cdot H_2O$ White to pale yellowish white powder. Very soluble in water, methanol or ethanol and sparingly soluble in ether or chloroform.

Water: 7.0 to 11.5% (0.5 g, volumetric titration, direct titration).

Content: NLT 95.0% (calculated on the anhydrous basis).

Assay: Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid hydrate, dissolve in 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.82 mg of $C_{10}H_8O_3S$

1-Naphthalenesulfonic acid $C_{10}H_8O_3S$

2-Naphthalenesulfonic acid See 2-naphthalene sulfonic acid Hydrate.

α-Naphthol benzein TS See p-naphtholbenzeine TS.

1-Naphthol $C_{10}H_7OH$ [Special class] Preserve in light-resistant containers.

1-Naphthol TS Dissolve 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in water to make 100 mL. Dissolve 1 g of 1-naphthol in this solution. Prepare before use.

2-Naphthol $C_{10}H_7OH$ [Special class] Preserve in light-resistant containers.

2-Naphthol TS Dissolve 1 g of 2-naphthol in sodium carbonate TS to make 100 mL. Prepare before use.

α-Naphthol See 1-naphthol.

α-Naphthol TS See 1-naphthol TS.

β-Naphthol See 2-naphthol.

β-Naphthol TS See 2-naphthol TS.

1-Naphthol-sulfuric acid TS Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid to mix. Prepare before use.

***p*-Naphtholbenzein** $C_{27}H_{20}O_3$ [Special class]

***p*-Naphtholbenzein TS** Dissolve 0.2 g of *p*-naphtholbenzein in acetic acid (100) to make 100 mL.

α-Naphtholbenzein See p-naphtholbenzein TS.

Purity *Clarity and color of solution*: Dissolve 0.1 g of sodium *β*-naphthoquinonesulfonate in 100 mL of ethanol (95); the resulting solution exhibits a red color and is clear.

Sensitivity: To 0.2 mL of a solution of sodium *β*-naphthoquinonesulfonate in ethanol (95) (1 in 1000), add 100 mL of freshly boiled and cooled water, and add 0.1 mL of 0.1 mol/L sodium hydroxide TS; the resulting solution exhibits a green color. Add subsequently 0.2 mL of 0.1 mol/L hydrochloric acid TS; the color of the resulting solution changes to yellowish red.

Potassium 1,2-naphthoquinone-4-sulfonate $C_{10}H_5O_2SO_3K$ [Special class]

Potassium 1,2-naphthoquiaone-4-sulfonate TS Dissolve 0.5 g of potassium 1,2-naphthoquiaone-4-sulfate in water to make 100 mL. Prepare before use.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate** $C_{18}H_{24}N_2O_2 \cdot 1/2H_2O$ [*N'*-diethylethylenediamine oxalate, special class] Preserve in a light-resistant container.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS** Dissolve 1 g of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate in water to make 1000 mL.

***N*-(1-Naphthyl)ethylenediamine dihydrochloride** $C_{12}H_{15}N_2$ [Special class]

***N*-(1-Naphthyl)ethylenediamine hydrochloride TS** Dissolve 100 mg of N-(1-naphthyl)ethylenediamine hydrochloride in a mixture of acetone and water (7 : 3) to make 100 mL.

1-Naphthylamine $C_{10}H_7NH_2$ [Special class] Preserve in light-resistant containers.

***α*-Naphthylamine** See 1-naphthylamine.

***N*-1-Naphthylethylenediamine dihydrochloride** $C_{10}H_7NH_2 \cdot 2HCl$ [Special class]

***N*-1-Naphthylethylenediamine dihydrochloride TS** Dissolve 0.1 g of *N*-1-naphthylethylenediamine dihydrochloride in a mixture of acetone and water (7 : 3).

***N*-1-Naphthylethylenediamine hydrochloride TS** See N-(1-naphthyl)ethylenediamine hydrochloride TS.

Nessler's TS To 10 g of potassium iodide, add 10 mL of water, and add saturated mercury(II) chloride solution until the red precipitate almost dissolves. To this solution, add a solution obtained by dissolving 30 g of potassium hydroxide in 60 mL of water, and then add 1 mL of saturated mercury(II) chloride solution and water to make 200 mL. After the precipitate settles, use the clear supernatant. Add 2 mL of this solution to 100 mL of ammonium chloride solution (1 in 3000000); the resulting solution immediately exhibits yellowish brown.

Neutral alumina for chromatography Prepared for chromatography (particle size between 75 and 180 μm).

Neutral alumina for column chromatography Prepared for column chromatography.

Neutral aluminum, 4% enhydrous Dry neutral alumina for column chromatography at 105 ℃ for 2 hours, transfer 50 g of it to a tight container, add 2.0 mL of water, shake well for homogeneous mixing, and then allow to stand for at least 2 hours.

Neutral detergent Synthetic detergent containing anionic or non-ionic surfactant. The pH of the 0.25% solution is 6.0 to 8.0. Add water for dilution to an appropriate concentration before use.

Neutral hydroxylamine TS See hydroxylamine TS, neutral.

Neutral red TS Dissolve 0.1 g of neutral red in acetic acid (100) to make 100 mL.

Neutral red [Special class]

Neutralized ethanol See ethanol, neutralized.

Neutralized methanol Add 3 drops of 1% bromophenol blue-methanol solution to 100 mL of methanol. If necessary, add 0.1 mol/L methanolic hydrochloric acid TS (12 mol/L hydrochloric acid TS diluted with 8 mL of methanol to make 1 L) until the color of the solution turns to yellow. Continue the dropwise addition until the color of the solution turns to bluish purple (deltamethrin, pyrazinobutazone).

Nicotinamide $C_6H_6N_2O$ [See monograph, Part I]

***β*-Nicotinamide adenine dinucleotide (*β*-NAD)** $C_{21}H_{27}N_7O_{14}P_2$ [*β*-NAD +]

Content: NLT 94.5%.

Assay: Weigh accurately about 25 mg of Sodium *β*-naphthoquinonesulfonate, and dissolve in water to make exactly 25 mL. Pipet 0.2 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the test solution. Perform the test with the test solution and 0.1 mol/L phosphate buffer solution, pH 7.0 as directed under the Ultraviolet-visible Spectroscopy using water as the blank, and determine the absorbances, A_T and A_S, at a wavelength of 260 nm.

$$\text{Amount of Nicotinamide adenine dinucleotide } (C_{21}H_{27}N_7O_{14}P_2) = \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_T - A_S) \times 25$$

***β*-Nicotinamide adenine dinucleotide (*β*-NAD) TS** Dissolve 40 mg of *β*-nicotinamide adenine dinucleotide in 10 mL of water. Prepare before use.

Nifedipine [See monograph, Part I]

Ninhydrin [Special class]

Ninhydrin solution Dissolve 0.4 g of ninhydrin in 10 mL of ethylene glycol monoethyl ether and add 10 mL of citric acid buffer solution (Ibuprofen lysine).

Ninhydrin solution Weigh 1 g of ninhydrin, and add 1-butanol to make 100 mL (calcium pantothenate).

Ninhydrin TS Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

Ninhydrin TS Dissolve 1.0 g of ninhydrin in ethanol, then add 1 mL of acetic acid and ethanol to make 100 mL (L-aspartate-L-arginine).

Ninhydrin TS Dissolve 2 g of ninhydrin in a mixture of citric acid buffer solution, pH 5 and 0-2-methoxyethanol (1 : 1) to make exactly 100 mL (methylolcefalexin lysinate).

Ninhydrin-ascorbic acid TS Dissolve 0.25 g of ninhydrin and 10 mg of L-ascorbic acid in water to make 50 mL. Prepare before use.

Ninhydrin-citric acid-acetic acid TS Dissolve 70 g of citric acid monohydrate in 500 mL of water, add 58 mL of acetic acid (100), 70 mL of a solution of sodium hydroxide (21 in 50) and water to make 1000 mL. Dissolve 0.2 g of ninhydrin in 100 mL of this solution.

Ninhydrin-ethanol solution To 0.1 g of ninhydrin, add 70 mL of anhydrous ethanol, and then add 2.9 mL of 2,4,6-trimethylpyridine and 20 mL of acetic acid (100) to mix.

Ninhydrin-stannous chloride TS See ninhydrin-tin(II) chloride TS.

Ninhydrin-sulfuric acid TS Dissolve 0.1 g of ninhydrin in 100 mL of sulfuric acid. Prepare before use.

Ninhydrin-tin(II) chloride TS To 200 mL of a solution obtained by dissolving 21.0 g of citric acid monohydrate in water, add sodium hydroxide TS to adjust the pH to 5.6 ± 0.2, add water to make 500 mL, and then dissolve in 1.3 g of tin(II) chloride dihydrate. To 50 mL of this solution, add 50 mL of a solution of ninhydrin in 2-methoxyethanol (2 in 50). Prepare before use.

Ninhydrin-water saturated 1-butanol TS, 0.2% Dissolve 2 g of ninhydrin in water saturated 1-butanol to make 1000 mL.

0.2% Ninhydrin-water saturated 1-butanol TS Dissolve 2 g of ninhydrin in water saturated 1-butanol to make 1000 mL.

Nitric acid HNO_3 [Special Class, specific gravity about 1.42]

Nitric acid, dilute Dilute 10.5 mL of nitric acid with water to make 100 mL (10%).

Nitric acid, fuming [Fuming nitric acid, Special Class, specific gravity about 1.5].

Nitric oxide–nitrogen dioxide detector column Melt-sealed glass column designed so that an appropriate adsorption filter, oxidation layer, and gas containing diphenylbenzidine (indicator), for detection of nitrogen monoxide and nitrogen dioxide, can pass through (determination range of nitrogen monoxide and nitrogen dioxide: 0.5 to 10 ppm)

2,2',2″-Nitrilotriethanol $(CH_2CH_2OH)_3N$ [First class]

4-Nitroaniline-sodium nitrite TS To 90 mL of a solution obtained by dissolving 0.3 g 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS, add 10 mL of sodium nitrite solution (1 in 20), and shake well to mix. Prepare before use.

***p*-Nitroaniline-sodium nitrite TS** See 4-nitroaniline-sodium nitrite TS.

4-Nitroaniline $C_6H_4NO_2NH_2$ Yellow to yellowish red crystal or crystalline powder.

Melting point 147 ℃ to 150 ℃.

Packaging and storage: Preserve in a light-resistant, tight container.

***p*-Nitroaniline** See 4-nitroaniline.

2-Nitrobenzaldehyde $O_2NC_6H_4CHO$ Pale yellow crystal or crystalline powder.

Melting point: 42 ℃ to 44 ℃

***o*-Nitrobenzaldehyde** See 2-nitrobenzaldehyde.

Nitrobenzene $C_6H_5NO_2$ [Special class]

4-Nitrobenzenediazonium chloride TS Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, and add a solution, prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while adding 1.5 mL of water to cool. Prepare before use.

4-Nitrobenzenediazonium chloride TS, for spraying Dissolve 0.4 g of 4-nitroaniline in 60 mL of 1 mol/L hydrochloric acid TS, add 1.5 mL of water to cool, and add sodium nitrite TS until the color of the potassium Iodide starch paper exhibits a blue color. Prepare before use.

***p*-Nitrobenzenediazonium chloride TS** See 4-nitrobenzenediazonium chloride TS.

***p*-Nitrobenzenediazonium chloride TS, for spraying** See 4-

nitrobenzenediazonium chloride TS, for nebulization.

4-Nitrobenzenediazonium chloride TS for spraying See 4-nitrobenzenediazonium chloride TS, for spraying.

***p*-Nitrobenzenediazonium chloride TS for spraying** See 4-nitrobenzenediazonium chloride TS, for spraying.

4-Nitrobenzenediazonium fluoroborate $O_2NC_6H_4N_2BF_4$ Pale yellowish white powder having almost no odor. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly soluble in ethanol (95) or chloroform.
To 10 mL of an aqueous solution of 4-nitrobenzenediazonium fluoroborate (1 in 1000), add 1 mL of phenol solution (1 in 1000) and 1 mL of sodium hydroxide TS; the resulting solution exhibits a red color.
Melting point: About 148℃ (with decomposition)
Loss on drying: NMT 1.0% (1 g, silica gel, 2 hours).

***p*-Nitrobenzenediazonium fluoroborate** See 4-nitrobenzenediazonium fluoroborate.

4-Nitrobenzoyl chloride $O_2NC_6H_4COCl$ Pale yellow crystal.
Melting point: 70 ℃ to 74 ℃
Content: NLT 98.0%.
Assay: Weigh accurately about 0.5 g of 4-nitrobenzoyl chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash the residue with water, dry at 105 ℃ to a constant mass, and then weigh the mass. Multiply the mass by 1.107 and make the amount of $C_7H_4ClNO_3$.

***p*-Nitrobenzoyl chloride** See 4-nitrobenzoyl chloride.

***p*-Nitrobenzyl bromide** See 4-nitrobromobenzyl.

4-Nitrobenzyl chloride $O_2NC_6H_4CH_2Cl$, Pale yellow crystal or crystalline powder. Soluble in ethanol.
Melting point: 71 ℃ to 73 ℃
Content: NLT 98.0%.
Assay: Weigh accurately about 0.5 g of 4-nitrobenzyl chloride, add 15 mL of a solution, prepared by dissolving 4 g of silver nitrate in 10 mL of water and adding ethanol to make 100 mL, and heat on a water bath under a reflux condenser for 1 hour. After cooling, filter the precipitate with a glass filter, wash the residue with water, dry at 105 ℃ to a constant mass, and weigh the mass. Use this mass as the amount of silver chloride (AgCl: 143.32).

Amount (mg) of 4-nitrobenzyl chloride ($C_7H_6ClNO_2$)
= Amount (mg) of silver chloride × 1.197

***p*-Nitrobenzyl chloride** See 4-nitrobenzyl chloride.

4-(4-Nitrobenzyl)pyridine $C_{12}H_{10}N_2O_2$ Pale yellow, crystalline powder. Freely soluble in acetone and soluble in ethanol (95).
Melting point: 69 ℃ to 71 ℃

4-(*p*-Nitrobenzyl)pyridine $C_{12}H_{10}N_2O_2$ Yellow crystal. Soluble in acetone. Dissolve 1 g of 4-(p-Nitrobenzyl)pyridine in acetone; it is soluble and clear.
Melting point: 71 ℃ to 74 ℃

4-Nitrobromobenzyl $NO_2C_6H_4CH_2Br$ Almost white to pale yellow crystal, which changes to black when exposed to light. Practically insoluble in water and freely soluble in ethanol and acetic acid (100). Preserve in light-resistant, tight containers.
Melting point: 98 ℃ to 100 ℃
Purity *Clarity and color of solution*: Dissolve 200 mg of 4-Nitrobromobenzyl in 5 mL of ethanol and 5 mL of acetic acid (100); the resulting solution is clear.

Nitroethane C2H5NO2
Density: Between 1.048 and 1.053 g/cm3 (20 °C)
Water: NMT 0.1%.Nitroethane C2H5NO2
Density: Between 1.048 and 1.053 g/cm3 (20 °C)
Water: NMT 0.1%.

Nitrogen N_2 [See monograph, Part II]

Nitrogen monoxide NO A colorless gas. Prepare by adding sodium nitrite TS to a solution of iron(II) sulfate heptahydrate in dilute sulfuric acid. Nitrogen monoxide from a pressure-resistant, hermetic containers may be used.

Nitromethane CH_3NO_2 [Special class]

2-Nitrophenyl-*β*-D-galactopyranoside $C_{12}H_{15}NO_8$ White crystalline powder, having no odor. Sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in ether.
Melting point: 193 ℃ to 194 ℃
Purity *Clarity and color of solution*: Dissolve 0.1 g of 2-nitrophenyl-*β*-D-galactopyranoside in 10 mL of water; the resulting solution is clear and colorless.
Loss on drying: NMT 0.1% (0.5 g, 105 ℃, 2 hours).
Content: NLT 98.0%.
Assay: Weigh accurately about 50 mg of 2-nitrophenyl-*β*-D-galactopyranoside, previously dried, and dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, and add water to make exactly 50 mL. Determine the absorbance A of this solution at 262 nm as directed under the Ultraviolet-visible Spectroscopy.

$$\text{Amount of 2-Nitrophenyl-β-D-galactopyranoside (mg)} = \frac{A}{133} \times 25000$$

***o*-Nitrophenyl-*β*-D-galactopyranoside** See 2-nitrophenyl-β-D-galactopyranoside.

1-Nitroso-2-naphthol $C_{10}H_7NO_2$ Yellowish brown to reddish brown crystalline powder.
Melting point 106 ℃ to 110 ℃
Packaging and storage: Preserve in a light-resistant tight container.

1-Nitroso-2-naphthol TS Dissolve 60 mg of 1-nitroso-2-naphthol in 80 mL of acetic acid (100), and add water to make 100 mL.

1-Nitroso-2-naphthol-3,6-disulfonic acid disodium $C_{18}H_5NNa_2O_8S_2$ [Special class]

***α*-Nitroso-*β*-naphthol TS** See 1-nitroso-2-naphthol TS.

Nitrous oxide N_2O A colorless and odorless gas. Use nitrous oxide from a pressure-resistant metal container.

***NN* indicator** Mix 0.5 g of 2-oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid and 50 g of anhydrous sodium sulfate, and grind them until the mixture becomes homogeneous.

Nonylphenoxy poly(ethyleneoxy) ethanol for gas chromatography Prepared for gas chromatography.

Normal agar medium Dissolve 25 g to 30 g of agar in 1000 mL of normal broth by heating. Replenish evaporated water, adjust the pH to between 6.4 and 7.0, filter, dispense the filrate, and sterilize with a high-pressure steam. Use the powdered agar in 15 g to 20 g.

Normal broth Dissolve 5 g of meat extract and 10 g of peptone in 1000 mL of water by warming gently, sterilize, and adjust the pH to between 6.4 and 7.0. After cooling, replenish evaporated water, and filter. Sterilize this solution with a high-pressure steam at 121 ℃ for 30 minutes.

Normal saline solution See isotonic sodium chloride injection.

***n*-Octadecane** $C_{18}H_{38}$ Colorless to white solid at ordinary temperature.

Purity *Clarity and color of solution*: A solution of *n*-Octadecane in chloroform (1 in 25) is colorless and clear.

Octadecyl silica gel monolithic packing for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized silica gel for liquid chromatography See octadecylsilanized silica gel, for liquid chromatography.

Octadecylsilanized silica gel for pretreatment See octadecylsilyl silica gel, for pretreatment.

Octadecylsilanized silica gel, for pretreatment Prepared for pretreatment.

Octadecylsilyl porous glass for liquid chromatography Prepared for liquid chromatography.

Octadecylsilyl porous silica gel for liquid chromatography Prepared for liquid chromatography.

Octane, iso Colorless liquid, practically insoluble in water. It is miscible with ether or chloroform.

Purity: Determine the absorbance of Octane, iso using water as the blank at the wavelengths of 230 nm, 250 nm and 280 nm; it is NMT 0.050, 0.010 and 0.005, respectively.

***n*-Octane** C_8H_{18}

Specific gravity d_4^{20}: 0.700 to 0.705

Purity: Perform the test with 2 µL of Octane, iso as directed under the Gas Chromatography according to the operating conditions in the Assay under Hypromellose. Determine each peak area by the automatic integration method, and calculate the amount of *n*-octane according to the percentage peak area method: the result is NLT 99.0%.

1-Octanol $CH_3(CH_2)_6CH_2OH$ [*n*-octyl alcohol, Special Class]

Octyl alcohol See 1-octanol.

***n*-Octylbenzene** $C_{14}H_{22}$ Clear and colorless liquid having a characteristic odor.

Specific gravity d_4^{20}: 0.854 to 0.863

Distilling range: 263 ℃ to 265 ℃, NLT 95 vol%

Octylsilane porous silica gel for liquid chromatography Prepared for liquid chromatography.

Octylsilane silica gel for liquid chromatography Prepared for liquid chromatography.

Olive oil [See monograph, Part II].

Olive oil emulsion Place 200 to 300 mL of a mixture of 3 volumes of polyvinyl alcohol TS and 1 volume of olive oil in a 500 mL container of an emulsifier, cool it to NMT 10 ℃ , and start emulsification at 12000 to 16000 rpm for 10 minutes. Allow the emulsified emulsion to stand for 1 hour in a cold place. Then, confirm that the oil layer is not separated and then use the emulsion (newlase).

Olive oil emulsion To 20 mL of neutralized olive oil, add 165 mL of 10 w/v% gum arabic solution (the clear supernatant taken after centrifuge is used, and store in a plastic container at -20 ℃) and 15 g of small ice pieces, and shake it at a high speed for emulsification. The emulsified particle has the size of 2 to 3 µm when observed with a microscope (pancreatin).

Orange II TS Weigh 100 mg of orangeII, place in 50 mL of phosphate buffer solution (pH 3.8 or 3.6) and warm it to dissolve. Once cooled, add buffer solution to make 100 mL (pentoxyverine citrate).

Orcin $C_7H_3O_2$ White to pale redish brown crystals or crystalline powder, having an unpleasant odor. It turns to red in color when oxidized in air. Soluble in water, ethanol (95), or ether.

Melting point: 107 ℃ to 111 ℃

Orcin-ferrous chloride TS Add 5 drops of 10% ferrous chloride solution to 0.2 g of orcin and add 100 mL of hydrochloric for mixing (glucuronolactone).

Orcin-ferrous chloride TS See orcin-Iron(III) chloride TS.

Orcin-Iron(III) chloride TS Add 10 mg of orcin to 1 mL of a solution of Iron(III) chloride hexahydrate in hydrochloric acid (1 in 1000) to dissolve. Prepare before use.

Osmium tetroxide O_SO_4 Colorless or pale yellow hygroscopic crystals or crystalline granule. Has a highly irritating odor. Is decomposed by light. Dissolves in ethanol or ether, and dissolves slowly in water.

Purity (1) *Clarity and color of solution:* Dissolve 0.2 g of osmium tetroxide in 1 mL of carbon tetrachloride; the solution exhibits a pale yellow color, is clear, and does not produce insoluble residue.

(2) *Non-volatile substance:* Evaporate the solution obtained from (1) to dryness on a water bath under a well-ventilated hood, and dry at 105 ℃ for 1 hour; the residue is NMT 0.4 mg.

(3) *Heavy metals:* To the residue in (2), add 2 mL of hydrochloric acid, and evaporate to dryness. Dissolve this residue in a small amount of water, add water to make 25 mL, and add hydrogen sulfide TS; the color of this soultion is not more intense

than the control solution. Prepare the control solution by adding water to 1.0 mL of lead standard solution to make 25 mL and adding 10 mL of hydrogen sulfide TS (NMT 50 ppm).

Note: The vapor of osmium tetroxide is toxic and highly irritating to eyes anc respiratory mucosa.

Oxalate pH standard solution See Oxalate pH Standard Solution in the pH Measurement under the General Tests.

Oxalic acid See oxalic acid dihydrate.

Oxalic acid dihydrate $H_2C_2O_4 \cdot 2H_2O$ [Special Class]

Oxalic acid *N*-(1-naphthyl)-*N′*-diethylethylenediamine $C_{18}H_{24}N_2O_2 \cdot 1/2H_2O$ [*N*-1-naphthyl-*N′*-diethylethylenediamine oxalate, Special Class] Store away from light.

Oxalic acid N-(1-naphthyl)-N'-diethylethylenediamine See N-(1-naphthyl)-N'-naphthylethylenediamine oxalate.

Oxalic acid *N*-(1-naphthyl)-*N′*-diethylethylenediamine TS Dissolve 1 g of oxalic acid *N*-(1-naphthyl)-*N′*-diethylethylenediamine in water to make 1000 mL.

Oxalic acid *N*-(1-naphthyl)-*N′*-diethylethylenediamine-acetone TS Dissolve 1 g of oxalic acid *N*-(1-naphthyl)-*N′*-diethylethylenediamine in a mixture of acetone and water (1 : 1) to make 100 mL. Prepare before use.

Oxalic acid TS Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

Oxidation solution Dissolve 10 g of chromium trioxide (CrO_3) in 80 mL of water, cool and shake. Add carefully 9 mL of sulfuric acid and add water to make 100.0 mL.

Oxygen O_2 [See monograph, Part I]

8-Oxyquinoline See 8-quinolinol.

Palladium chloride See palladium(II) chloride.

Palladium chloride TS Dissolve palladium chloride 200 mg with hydrochloric acid 2 mL and add water to make 100 mL. Take 25 mL of this solution and add 1 mol/L sodium acetate TS 50 mL, acetone 20 mL, 1 mol/L hydrochloric acid 48 mL and water to make 250 mL (promethazine hydrochloride).

Palladium chloride TS See palladium(II) chloride TS.

Palladium(II) chloride $PdCl_2$ [Special Class]

Palladium(II) chloride TS Dissolve 0.2 g of palladium(II) chloride with 500 mL of 0.25 mol/L sulfuric acid TS, heat if necessary, and cool it. Then, add 0.25 mol/L sulfuric acid TS to make 1000 mL.

Palmatine chloride See palmatine chloride.

Palmatine hydrochloride hydrate $C_{21}H_{22}ClNO_4 \cdot nH_2O$ It is a yellowish brown crystalline powder.

Purity: Weigh 1 mg of palmatine hydrochloride Hydrate, dissolve in methanol to make exactly 10 mL, and use this solution as the test solution. Take 20 μL of this solution, and perform the test as directed under the Assay for Phellodendri Cortex, and then perform the test according to the Liquid Chromatography. The total peak area other than that of palmatine in the test solution is NMT one tenth of the total peak area divided by the peak area of the solvent.

Pancreatic digestion of casein Casein that has undergone enzymatic hydrolysis with pancreatin, meeting the following specifications:

Description: Grayish yellow powder, having a characteristic odor. The color turns to lemon yellow when dissolved in water.

Nitrogen	NLT 10%
Amino acid/total nitrogen (%)	25 to 50%
Loss on drying	NMT 7%
Residue on ignition	NMT 15%

Papaic digest of soybean Soybean protein that has undergone zymolysis using papain.

Papaverine hydrochloride $C_{20}H_{21}NO_4 \cdot HCl$ [See monograph, Part I]

Paraffin [See monograph, Part II].

Paraffin, liquid [See monograph, Part II on Light Liquid Paraffin].

Peanut oil [See monograph, Part II]

Penta ethylenehexaaminated polyvinyl alcohol polymer for liquid chromatography Prepared for liquid chromatography.

Pentadecanoic acid $CH_3(CH_2)_{13}COOH$

Pentane $CH_3(CH_2)_3CH_3$ A clear and colorless liquid.
Specific gravity d_{20}^{20}: 0.620 to 0.630
Distilling range: 35.5 to 37 ℃, NLT 98 vol%.

Peppermint oil [See monograph, Part II]

Pepsin, Saccharated See Saccharated pepsin.

Peptone Prepared for microbial test.

Peptone, animal tissue Prepared for microbial test.

Peptone, casein A grayish yellow powder, having a characteristic but not putrescent odor. It dissolves in water, but not in ethanol (95) or ether.

Degree of digestion: Dissolve 1 g of Sodium 1-Pentane Sulfonate in 10 mL of water, and perform the following test using this solution as the test solution.

(1) To 1 mL of the test solution, overlay 0.5 mL of a mixture of 10 mL of dilute ethanol and 1 mL of acetic acid (100); no ring or precipitate forms at the junction of the two liquids. Also, this solution does not become cloudy when shaken to mix.

(2) Mix 1 mL of the test solution with 4 mL of a saturated solution of zinc sulfate; a small quantity of precipitate is produced (proteoses).

(3) Filter the mixture of (2), and to 1 mL of the filtrate, add 3 mL of water and 4 drops of bromine TS; a reddish purple color is produced.

Loss on drying: NMT 7% (0.5 g, 105 ℃, constant mass).
Residue on ignition: NMT 15% (0.5 g).
Nitrogen content: NLT 10% (105 ℃, constant mass, after

drying, according to the Nitrogen Determination).

Peptone, diced Prepared for microbial test.

Peptone, gelatin Prepared for microbial test.

Peptone, soybean Prepared for microbial test.

Perchloric acid $HClO_4$ [Special class, density: about 1.67 g/mL, concentration: 70 to 72%]

Perchloric acid TS To 8.5 mL of perchloric acid, add water to make 100 mL.

Perchloric acid-anhydrous ethanol TS See perchloric acid-ethanol (99.5) TS.

Perchloric acid-ethanol (99.5) TS Carefully add 25.5 mL of perchloric acid to 50 mL of ethanol (99.5), cool, and then add ethanol (99.5) to make 100 mL (3 mol/L).

Peroxidase It is obtained from horseradish, and is a reddish-brown powder. Fast Blue B Salt is freely soluble in water. Each mg of Fast Blue B Salt contains about 250 units. However, 1 unit of Fast Blue B Salt is the amount of enzyme that produces 1 mg of purpurogallin in 20 seconds at pH 6.0 and 20。C using pyrogallol and hydrogen peroxide (30) as substrates.

Peroxidase-labeled Bradykinin To prepare it, obtain peroxidase from horseradish, bond it with bradykinin, and dissolve in gelatin-phosphate buffer solution (pH 7.0). The resulting solution is a colorless to pale brown, clear liquid.

Peroxidase-labeled Bradykinin TS To 0.08 mL of peroxidase-labeled bradykinin, 8 mg of sodium tetraborate decahydrate, 8 mg of bovine serum albumin, and 0.8 mL of gelatin-phosphate buffer solution (pH 7.0), add water to make 8 mL, and freeze-dry. Add 8 mL of water to dissolve. Prepare this solution Prepare before use.

Petrolatum [See monograph, Part II on White Petrolatum or Yellow Petrolatum].

Petroleum benzene [Special class]

Petroleum ether [Special class]

Petroleum-based hexamethyltetracosane branched hydrocarbon mixture for gas chromatography (L) Prepared for gas chromatography.

Phenacetin $C_{10}H_{13}NO_2$ [First class]

1,10-Phenanthroline hydrochloride monohydrate $C_{12}H_8N_2 \cdot HCl \cdot H_2O$ [Special class]

***o*-Phenanthroline hydrochloride** See 1,10-Phenanthroline hydrochloride monohydrate.

1,10-Phenanthroline monohydrate $C_{12}H_8N_2 \cdot H_2O$ [Special class]

1,10-Phenanthroline monohydrate TS Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of newly prepared iron(II) sulfate heptahydrate solution (37 in 2500) and 1 mL of dilute sulfuric acid. Cap and preserve.

Phenanthroline solution Weigh 2.5 g of o-phenanthroline, and dissolve it in 100 mL of water and 900 mL of ethanol.

***o*-Phenanthroline** See 1,10-Phenanthroline monohydrate.

***o*-Phenanthroline TS** See 1,10-Phenanthroline monohydrate TS

Phenethylamine Hydrochloride $C_6H_5CH_2CH_2NH_2 \cdot HCl$ White crystals or crystalline powder.
Melting point: 220 to 225℃

Phenobarbital sodium $C_{12}H_{11}N_2NaO_3$ [See monograph, Part I]

Phenol C_6H_5OH [Special class]

Phenol Red $C_{19}H_{14}O_5S$ [Special class]

Phenol red TS Dissolve 100 g of phenol red in 100 mL of ethanol (95). Filter, if necessary.

Phenol red TS, dilute To 235 mL of dilute ammonium nitrate solution (1 in 9400), add 105 mL of 2 mol/L sodium hydroxide TS and 135 mL of a solution prepared by dissolving 24 g of acetic acid (100) in 200 mL of water, and mix. To this solution, add 25 mL of a solution prepared by dissolving 33 mg of phenol red in 1.5 mL of 2 mol/L sodium hydroxide TS and adding water to make 100 mL. If necessary, adjust the pH to 4.7.

Phenol-fuchsin solution Weigh 11 g of fuchsin, dissolve in 100 mL of ethanol (99.5), add 100 mL of phenol solution (1 in 200), shake to mix, filter, and use the filtrate (activated lactobacillus sporogenes).

Phenolphthalein $C_{20}H_{14}O_4$ [Special class]

Phenolphthalein TS Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

Phenolphthalein-thymol blue TS Solution A: Dissolve 0.1 g of phenolphthalein in 100 Ml of diluted ethanol (95) (4 in 5). Solution B: Dissolve 0.1 g of thymol blue in 50 mL of a mixture of ethanol and dilute sodium hydroxide TS (250 : 11), and add water to make 100 mL. Mix 2 volumes of solution A and 3 volumes of solution B immediately prior to use.

Phenyl benzoate $C_{13}H_{10}O_2$ White crystals or crystalline powder, having a slightly characteristic odor.
Melting point: 68 ℃ to 70 ℃
Purity: *CClarity and color of solution:* Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol; the solution is clear.

Phenyl porous silica for liquid chromatography Prepared for liquid chromatography.

Phenyl silica for liquid chromatography Prepared for liquid chromatography.

Phenyl silica gel for liquid chromatography Prepared for liquid chromatography.

1-Phenyl-3-methyl-5-pyrazolone See 3-methyl-1- phenyl-5-

pyrazolone.

25%Phenyl-25% cyanopropylmethyl silicone polymer for gas chromatography Prepared for gas chromatography.

5% Phenyl-95% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

50% Phenyl-50% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

75% Phenyl-25% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

Phenylalanine $C_9H_{11}NO_2$ [See monograph, Part I on L-phenylalanine]

***o*-Phenylenediamine Dihydrochloride** $H_2NC_6H_4NH_2$ White to dark brown crystals or crystalline powder, freely soluble in ethanol or acetone and soluble in water.

Content: NLT 95% Assay Weigh accurately about 0.15 g of o-phenylenediamine dihydrochloride, dissolve in 50 mL of acetic acid for non-aqueous titration, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration under Titrimetry). Perform a blank test in the same manner and make any necessary correction.

Phenylfluorone $C_{19}H_{12}O_5$ [Special class]

Phenylfluorone-ethanol TS Weigh accurately 50 mg of phenylfluorone, dissolve in 10 mL of ethanol (95) and hydrochloric acid (1 in 3), and add ethanol (95) again to make exactly 500 mL.

Phenylglycine $C_6H_5CH(NH_2)COOH$ [*D*(-)-2-phenylglycine, First class]

Phenylhydrazine $C_6H_5NHNH_2$ [Special class]

Phenylhydrazine hydrochloride See phenylhydrazinium hydrochloride.

Phenylhydrazine hydrochloride TS See phenylhydrazinium hydrochloride TS.

Phenylhydrazinium ethanol TS Dissolve 1.8 g of phenylhydrazinium hydrochloride in 50 mL of water and 50 mL of 96% ethanol.

Phenylhydrazinium Hydrochloride $C_6H_5NHNH_2 \cdot HCl$ [Special class]

Phenylhydrazinium hydrochloride TS Weigh 65 mg of phenylhydrazinium hydrochloride, recrystallized from dilute ethanol, dissolve in 100 mL of a solution previously prepared by adding cautiously 170 mL of sulfuric acid to 80 mL of water.

5% Phenylmethylsilicone polymer for gas chromatography Prepared for gas chromatography.

35% Phenylmethylsilicone polymer for gas chromatography Prepared for gas chromatography.

50% Phenylmethylsilicone polymer for gas chromatography Prepared for gas chromatography.

65% Phenylmethylsilicone polymer for gas chromatography Prepared for gas chromatography.

Phloroglucin $C_6H_3(OH)_3 \cdot 2H_2O$ [Special class]

Phloroglucin-hydrochloric acid TS Dissolve 1 g of phloroglucin in 10 mL of ethanol, then add 40 mL of hydrochloric acid.

Phosgene paper Dissolve 5 g of 4-dimethylaminobenzaldehyde and 5 g of diphenylamine in 100 mL of ethanol (99.5), soak a 5 cm wide filter paper in this, hang the filter paper in clean air in the dark, and dry naturally. Cut off and discard the top and bottom ends of the filter strip by 5 cm, and cut the remaining parts into 7.5 cm long pieces to make filter strips. Preserve in light-resistant, tight containers. Do not use anything that has turned yellow.

Phosphatase TS, alkaline Add magnesium buffer solution, pH 9, to 95 ± 5 mg of alkaline phosphatase to make 50 mL. Prepare this test solution immediately prior to use.

Phosphatase, alkaline [First class]

Phosphate buffer solution Dissolve 1.36 g of potassium dihydrogen phosphate and 5 g of triethanolamine in 1000 mL of water and adjust the pH to 11.5 (oxolamine citrate syrup, oxolamine citrate tablet).

Phosphate buffer solution Dissolve 220 mg of disodium hydrogen phosphate heptahydrate and 800 mg of potassium dihydrogen phosphate in water to make 100 mL (azlocillin sodium).

Phosphate buffer solution Dissolve 23 g of phosphoric acid and 1.5 g of heptane sulfonate in water to make 1 L (S-carboxymethylcystein and sobrerol syrup).

0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 0.1 mol/L phosphate buffer solution, pH 8.0 Dissolve 16.73 g of disodium hydrogen phosphate for antibiotics and 0.523 g of potassium dihydrogen phosphate in 750 mL of water, add phosphoric acid to adjust the pH to 8.0, and add water to make 1000 mL.

Phosphate buffer solution for pancreatin See phosphate buffer solution, for pancreatin.

Phosphate buffer solution, 0.01 mol/L, pH 3.0 Take about 1.38 g of disodium hydrogen phosphate, dissolve in 800 mL of water, adjust the pH to 3.0 with 50% phosphoric acid, and add water to make 1000 mL.

Phosphate buffer solution, 0.01 mol/L, pH 7.0 To a solution prepared by adding water to 3.58 g of disodium hydrogen phosphate to make 1000 mL, add a solution prepared by dissolving 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, until the pH is adjusted to 7.0 (volume ratio: 2 : 1).

Phosphate buffer solution, 0.02 mol/L, pH 3.0 Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Phosphate buffer solution, 0.02 mol/L, pH 8.0 Add 300 mL of water to 50 mL of 0.2 mol/L potassium dihydrogen phosphate

TS, adjust the pH to 8.0 with sodium hydroxide TS, and add water to make 500 mL.

Phosphate buffer solution, 0.02 mol/L, pH 8.0 Add water to 100 mL of 0.1 mol/L phosphate buffer solution (pH 8.0) to make 500 mL (semi-alkaline protease).

Phosphate buffer solution, 0.05 mol/L To a solution prepared by dissolving 8.95 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, add a solution prepared by dissolving 3.4 g of potassium dihydrogen phosphate in water to make 500 mL, at a volume ratio of 2 : 1.

Phosphate buffer solution, 0.05 mol/L, pH 4.0 Dissolve 7.80 g of potassium dihydrogen phosphate in water, adjust the pH to 4.0 with phosphoric acid, and add water to make 1000 mL.

Phosphate buffer solution, 0.05 mol/L, pH 4.5 Add dilute phosphoric acid or dilute sodium hydroxide to 0.05 mol/L potassium dihydrogen phosphate solution to adjust the pH to 4.5.

Phosphate buffer solution, 0.05 mol/L, pH 6.0 Dissolve 6.81 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 6.0 to 6.1 with sodium hydroxide solution (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, 0.05 mol/L, pH 7.0 Dissolve 4.83 g of dipotassium hydrogen phosphate and 3.02 g of potassium dihydrogen phosphate in 1000 mL of water and adjust the pH to 7.0 with phosphoric acid or potassium hydroxide TS.

Phosphate buffer solution, 0.1 mol/L Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 mL of water.

Phosphate buffer solution, 0.1 mol/L, pH 4.0 Dissolve 15.5 g of sodium dihydrogen phosphate in 900 mL of water, adjust the pH to 4.0 with phosphoric acid, and add water to make 1000 mL.

Phosphate buffer solution, 0.1 mol/L, pH 4.5 Dissolve 13.6 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 4.4 to 4.6 with 1 mol/L potassium hydroxide TS. Then, add water to make 1000 mL.

Phosphate buffer solution, 0.1 mol/L, pH 6.0 Dissolve 6.0 g of disodium hydrogen phosphate and 7.0 g of potassium dihydrogen phosphate in about 750 mL of water by boiling for NLT 1 minute. If necessary, adjust the pH to 6.0 to 6.1 with sodium hydroxide TS or phosphoric acid. Then, add water to make 1000 mL.

Phosphate buffer solution, 0.1 mol/L, pH 6.0 Mix 880 mL of potassium dihydrogen phosphate solution (13.3 in 1000) and 120 mL of anhydrous sodium monohydrogen phosphate solution (14.2 in 1000). If necessary, adjust the pH to 6.0 with diluted phosphoric acid (Diastase and protease N1).

Phosphate buffer solution, 0.1 mol/L, pH 6.8 Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate dodecahydrate in about 750 mL of water. If necessary, adjust the pH to 6.8 to 6.9 with 1 mol/L sodium hydroxide TS. Then, add water to make 1000 mL.

Phosphate buffer solution, 0.1 mol/L, pH 7.0 Dissolve 10.65 g of disodium hydrogen phosphate and 3.40 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 6.9 to 7.1 with sodium hydroxide TS or phosphoric acid. Then, add water to make 1000 mL.

Phosphate buffer solution, 0.1 mol/L, pH 7.0 To a solution prepared by dissolving 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, add a solution prepared by dissolving 6.8 g of potassium dihydrogen phosphate in water to make 500 mL until the pH is adjusted to 7.0 (volume ratio: about 2 : 1).

Phosphate buffer solution, 0.1 mol/L, pH 8.0 To Solution A (35.8 g of Na_2HPO_4 mixed with water to make 1 mL), add Solution B (13.6 g of KH_2PO_4 dissolved in water to make 1 mL) to adjust the pH 8.0 (semi-alkaline protease).

Phosphate buffer solution, 0.1 mol/L, pH 8.0 (1) 16. 73 g of disodium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate, or (2) 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate. Prepare according to (1) or (2). In either of the cases, dissolve the specified amount in about 750 mL of water. If necessary, adjust the pH to 7.8 to 8.0 with phosphoric acid. Then, add water to make 1000 mL. (gentamicin sulfate implant)

Phosphate buffer solution, 0.1 mol/L, pH 8.0 Add potassium dihydrogen phosphate TS to 0.1 mol/L sodium monohydrogen phosphate TS to adjust the pH to 8.0 (pancreatic digestive enzyme TA).

Phosphate buffer solution, 0.1 mol/L, pH 8.0 Dissolve 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 8.0 with phosphoric acid. Then, add water to make 1000 mL.

Phosphate buffer solution, 0.1mol/L, pH 7.2 Dissolve 22.32g of sodium monohydrogen phosphate and 5.13 g of potassium dihydrogen phosphate in water to make 1 L. If necessary, adjust the pH to 7.2 with 5 mol/L sodium hydroxide TS.

Phosphate buffer solution, 0.2 mol/L, pH 10.5 Add 2.0 mL of 10 mol/L potassium hydroxide TS to 35.0 g of dibasic potassium phosphate, add about 750 mL of water to dissolve, adjust the pH to 10.4 to 10.6 with sodium hydroxide solution (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, 0.2 mol/L, pH 6.8 Mix 375 mL of 0.1 mol/L hydrochloric acid and 125 nL of 0.2 mol/L sodium phosphate TS and adjust the pH to 6.8 ± 0.05 with 2 mol/L hydrochloric acid or 2 mol/L sodium hydroxide TS.

Phosphate buffer solution, 0.2 mol/L, pH 6.8 Mix 510 mL of 0.2 mol/L potassium dihydrogen phosphate solution and 490 mL of 0.2 mol/L sodium monohydrogen phosphate TS and stir well (pancreatin).

Phosphate buffer solution, 0.2 mol/L, pH 6.8 Dissolve 13.88 g of potassium dihydrogen phosphate and 17.44 g of sodium dihydrogen phosphate dihydrate in 900 mL of water 900, adjust the pH to 6.8 with 0.1 mol/L sodium hydroxide solution or 0.1 mol/L hydrochloric acid, and then add water to make 1000 mL (pancrease).

Phosphate buffer solution, 0.2 mol/L, pH 7.0 See phosphate buffer solution, pH 7.0 (uridine).

Phosphate buffer solution, 0.2 mol/L, pH 8.0 Add 0.2 mol/L potassium dihydrogen phosphate TS to 0.2 mol/L disodium hydrogen phosphate TS to adjust the pH to 8.0.

Phosphate buffer solution, 0.21 mol/L, pH 4.0 Dissolve 6.80 g of potassium dihydrogen phosphate and 9.35 g of sodium chloride 9.35 g in 900 mL of water and adjust the pH to 4.0± 0.05 with 0.05 mol/L phosphoric acid solution.

Phosphate buffer solution, 0.5 mol/L, pH 7.0 Dissolve 26.5 g of potassium dihydrogen phosphate and 53.2 g of dibasic potassium phosphate in water to make 1000 mL.

Phosphate buffer solution, 1%, pH 6.0 (1) 2.0 g of potassium monohydrogen phosphate and 8.0 g of potassium dihydrogen phosphate, (2) 10.0 g of potassium dihydrogen phosphate, (3) 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate.
Prepare according to (1), (2) or (3). In any of the above cases, weigh the specified amount and add about 750 mL of water to dissolve. If necessary, adjust the pH to 5.9 to 6.1 with 1 mol/L potassium hydroxide TS for (1) and (3) or with sodium hydroxide solution (1 in 10) for (2), and then add water to make 1000 mL.

Phosphate buffer solution, 1%, pH 6.5 Dissolve 10 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 6.4 to 6.6 with sodium hydroxide solution (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, 1%, pH 8.0 Dissolve 10 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 7.9 to 8.1 with sodium hydroxide solution (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, 1/15 mol, pH 6.8 Mix 510 mL of 0.2 mol/L potassium dihydrogen phosphate solution and 490 mL of 0.2 mol/L sodium monohydrogen phosphate solution and shake well, and dilute to 3-fold (pancreatin).

Phosphate buffer solution, 1/15 mol, pH 7.0 Dissolve 4.5 g of potassium dihydrogen phosphate in water to make 500 mL and the resultant solution will be Solution A. Dissolve 4.73 g of anhydrous sodium monohydrogen phosphate in water to make 500 mL and the resultant solution will be Solution B. Mix 38.9 mL of Solution A and 61.0 mL of Solution B. If necessary, adjust the pH to 7.0 by slowly adding anhydrous sodium monohydrogen phosphate solution. (chymotrypsin for injection)

Phosphate buffer solution, 1/15 mol/L, pH 5.6 Dissolve 9.07 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 5.5 to 5.7 with 1 mol/L potassium hydroxide TS. Then, add water to make 1000 mL.

Phosphate buffer solution, 10%, pH 6.0 Dissolve 20.0 g of potassium monohydrogen phosphate and 80.0 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 6.0 to 6.1 with potassium hydroxide solution (1 in 10). Then, add water to make 1000 mL.

Phosphate buffer solution, 7.8 mmol/L, pH 6.6 Dissolve 1 mL of 85% phosphoric acid and 1 mL of 50% sodium hydroxide solution in water to make 1000 mL.

Phosphate buffer solution, for pancreatin Dissolve 3.3 g of anhydrous sodium monohydrogen phosphate, 1.4 g of potassium dihydrogen phosphate and 0.33 g of sodium chloride in water to make 100 mL.

Phosphate buffer solution, for washing microplates Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate dodecahydrate, 52.6 g of sodium chloride, 3.0 g of polysorbate 80 and 1.8 g of polyoxyethylene (40) octylphenyl ether in water to make 600 mL. To 1 volume of this solution, add 9 volumes of water before use.

Phosphate buffer solution, pH 11.0 To 50 mL of 0.1 mol/L sodium phosphate TS, add 8.26 mL of 0.1 mol/L sodium hydroxide TS, and then add water to make 100 mL.

Phosphate buffer solution, pH 12 To 5.44 g of anhydrous sodium monohydrogen phosphate, add 36.5 mL of sodium hydroxide TS and about 40 mL of water, dissolve by shaking well to mix, and add water to make 100 mL.

Phosphate buffer solution, pH 3.0 Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Phosphate buffer solution, pH 3.8 Add 400 mL of water to 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS, adjust the pH to 3.8 with 0.2 mol/L hydrochloric acid, and then add water to make 1 L.

Phosphate buffer solution, pH 5.2 Dissolve 9.072 g of potassium dihydrogen phosphate in water to make 1 L, and the resultant solution will be Solution A. Dissolve 4.753 g of sodium monohydrogen phosphate in water to make 200 L, and the resultant solution will be Solution B. Add Solution B to Solution A to adjust the pH to 5.2 (compound loperamide HCL, berberine chloride and acrinol capsule).

Phosphate buffer solution, pH 5.4 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS and 2.5 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL. If necessary, adjust the pH to 5.6 with nutrient solution.

Phosphate buffer solution, pH 5.8 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS and 3.6 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

Phosphate buffer solution, pH 6.0 Add 0.1 mol/L potassium dihydrogen phosphate to 0.1 mol/L sodium monohydrogen phosphate solution to adjust the pH to 6.0 (lipase).

Phosphate buffer solution, pH 6.0 Dissolve 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate in about 750 mL of water, adjust the pH to 6.0 with sodium hydroxide TS or diluted phosphoric acid (1 in 15) if necessary, and add water to make 1000 mL.

Phosphate buffer solution, pH 6.0 Mix 87.8 mL of 0.2 mol/L sodium dihydrogen phosphate and 12.3 mL of 0.2 mol/L sodium monohydrogen phosphate TS to adjust the pH to 6.0, and add water to make 200 mL.

Phosphate buffer solution, pH 6.0, 10% Dissolve 20.0 g of dibasic potassium phosphate and 80.0 g of potassium dihydrogen phosphate in about 750 mL of water. If necessary, adjust the pH to 6.0 to 6.1 with potassium hydroxide solution (1 in 10).

Then, add water to make 1000 mL.

Phosphate buffer solution, pH 6.2 To 800 mL of a 1 L solution prepared by dissolving 9.08 g of potassium dihydrogen phosphate in water, add 200 mL of a 1 L solution prepared by dissolving 11.9 g of anhydrous sodium monohydrogen phosphate in water. If necessary, adjust the pH to 6.2 with nutrient solution.

Phosphate buffer solution, pH 6.5 Dissolve 126 g of potassium dihydrogen phosphate in 700 mL of water, adjust the pH to 6.5 with 10% sodium hydroxide TS, and add water to make 1L.

Phosphate buffer solution, pH 6.5 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 15.20 mL of 0.2 mol/L sodium hydroxide solution, and add water to make 200 mL.

Phosphate buffer solution, pH 6.8 Dissolve 3.40 g of potassium dihydrogen phosphate and 3.35 g of anhydrous sodium monohydrogen phosphate with water to make 1000 mL.

Phosphate buffer solution, pH 6.9 Dissolve 6.8 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 6.9 with sodium hydroxide TS.

Phosphate buffer solution, pH 7.0

Solution A: Add water to 9.08 g of sodium dihydrogen phosphate to make 1 L.

Solution B: Add water to 11.88 g of sodium monohydrogen phosphate to make 1 L. Add Solution B to Solution A to adjust the pH to 7.0, then use the resultant solution (soluble azulene).

Phosphate buffer solution, pH 7.0 Dissolve 0.5 g of disodium hydrogen phosphate and 0.3 g of potassium dihydrogen phosphate in 1000 mL of water and add phosphoric acid to adjust the pH to 7.0 (sodium picosulfate tablet).

Phosphate buffer solution, pH 7.0 Dissolve 2.5 g of potassium dihydrogen phosphate and 4.1 g of sodium monohydrogen phosphate in water to make 1000 mL, and adjust the pH to 7.0 with phosphoric acid (erythromycin stinoprate).

Phosphate buffer solution, pH 7.0 Dissolve 6.97 g of potassium monohydrogen phosphate in 1 L of purified water and adjust the pH to 7.2 with 10% phosphoric acid solution (lercanidipine hydrochloride).

Phosphate buffer solution, pH 7.0 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 29.54 mL of 0.2 mol/L sodium hydroxide solution, and add water to make 200 mL.

Phosphate buffer solution, pH 7.0 Place 9.47 g of anhydrous sodium monohydrogen phosphate in a 1-L volumetric flask, add water to the gauge line, and then the resultant solution will be Solution A. Place 9.08 g of potassium dihydrogen phosphate in a 1-L volumetric flask, add water to the gauge line, and then the resultant solution will be Solution B. Mix Solution A and solution B at a of 612:388. (clemastine fumarate, clemastine fumarate injection).

Phosphate buffer solution, pH 7.2 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide solution, and add water to make 200 mL.

Phosphate buffer solution, pH 7.4 Dissolve 1.8 g of potassium dihydrogen phosphate, 7.57 g of anhydrous sodium monohydrogen phosphate and 0.035 g of calcium acetate in water to make 1 L (protease).

Phosphate buffer solution, pH 7.4 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 39.50 mL of 0.2 mol/L sodium hydroxide solution, and add water to make 200 mL.

Phosphate buffer solution, pH 7.4 Place 4.026 g of sodium monohydrogen phosphate and 0.0676 g of potassium dihydrogen phosphate in a 500-mL volumetric flask, add 450 mL of water to dissolve, adjust the pH to 7.4, and add water to fill to the gauge line. Sterilize the solution (streptokinase and streptodornase).

Phosphate buffer solution, pH 7.5 Add water to 6.8 g of potassium dihydrogen phosphate to make 250 mL, and then add 190 mL of 0.2 mol/L sodium hydroxide TS and water 400 mL. Adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS and then add water again to make 1 L (acetyl-L-carnitine hydrochloride).

Phosphate buffer solution, pH 7.5 Mix disodium hydrogen phosphate solution (53.7 in 1000) and potassium phosphate solution (20.4 in 1000) at a ratio of 21 : 4 (chlorophyllin copper complex sodium).

Phosphate buffer solution, pH 7.5 Place 34 mL of 85% phosphoric acid in a 10-L volumetric flask containing 5 L of water, add 40 mL of 50% sodium hydroxide solution, and then add water to the gauge line. Adjust the pH to 7.5 with 50% sodium hydroxide solution.

Phosphate buffer solution, pH 7.6 Mix 5.2 mL of 1 mol/L sodium dihydrogen phosphate TS and 63.2 mL of 0.5 mol/L sodium monohydrogen phosphate TS, and add water to make 4000 mL (omeprazole tablet).

Phosphate buffer solution, pH 7.6 To 500 mL of 0.25 mol/L sodium monohydrogen phosphate TS, add 0.25 mol/L potassium dihydrogen phosphate TS to adjust the pH to 7.6.

Phosphate buffer solution, pH 7.8 Dissolve 0.58 g of potassium dihydrogen phosphate and 8.86 g of anhydrous sodium monohydrogen phosphate in water to make 1 L, and adjust the pH to 7.8 with phosphoric acid (glimepiride).

Phosphate buffer solution, pH 8.0 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 46.1 mL of 0.2 mol/L sodium hydroxide solution, and add water to make 200 mL.

Phosphate buffer solution, pH 8.0 To a 1 L solution prepared by dissolving 35.8 g of sodium monohydrogen phosphate in water, add a 1 L solution prepared by dissolving 13.6 g of potassium dihydrogen phosphate in water until the pH is adjusted to 8.0.

Phosphate pH standard solution See pH Measurement under the General Tests.

Phosphate-acetate-borate buffer solution, pH 2.0 Dissolve 6.77 mL of phosphoric acid, 5.72 mL of acetic acid (100) and 6.18 g of boric acid 6.18 g in water to make 1000 mL. To this solution, add 0.5 mol/L sodium hydroxide TS to adjust the pH to

2.0.

Phosphenic acid H_3PO_4 [First class]

Phosphomolybdic acid See phosphomolybdic acid *n*-hydrate.

Phosphomolybdic acid *n*-hydrate $P_2O_5 \cdot 24MoO_3 \cdot nH_2O$ [12molybdo(VI)phosphoric acid *n*-hydrate, Special Class]

Phosphomolybdic acid TS Dissolve 1 g of phosphomolybdic acid in 10 mL of water and then add ethanol to make 100 mL.

Phosphomolybdic acid TS Dissolve 1.0 g of phosphomolybdic acid n-hydrate in ethanol (95) to make 10 mL. Prepare before use.

Phosphomolybdic acid-tungstic acid TS Add 50 g of sodium tungstate (VI), 12 g of molybdic acid and 25 mL of phosphoric acid into a round flask containing about 350 mL of water, shake to mix, reflux for 2 hours, cool, and add water to make 500 mL.

Phosphomolybdic acid-tungstic acid TS (for nifuroxazide purity test) To 700 mL of water, add 100 g of sodium tungstate and 25 g of sodium molybdate dihydrate to dissolve, add 100 mL of hydrochloric acid and 50 mL of phosphoric acid, and heat the solution for 10 hours under a reflux condenser. To this solution, add 150 g of lithium sulfate monohydrate, 50 mL of water and a few drops of bromine, and heat the resulting solution for about 15 minutes to eliminate excess bromide. After cooling, add water to make 1000 mL, and filter. The test solution is yellow in color. If greenish color appears, the solution is not suitable. In this case, the solution may be boiled with some additional drops of bromine for recycling.

Phosphoric acid H_3PO_4 [Special Class]

Phosphoric acid-sodium sulfate buffer solution, pH 2.3 Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water and add 2.7 mL of phosphoric acid. If necessary, adjust the pH to 2.3 with 2-aminoethanol.

Phosphorus pentoxide P_2O_5 [Special class].

Phosphorus pentoxide See phosphorus(V) oxide.

Phosphotungstic acid See phosphotungstic acid *n*-hydrate.

Phosphotungstic acid *n*-hydrate $P_2O_5 \cdot 24WO_3 \cdot nH_2O$ [12tungsten(VI)phosphoric acid *n*-hydrate, Special Class]

Phosphotungstic acid TS Dissolve 1 g of phosphotungstic acid *n*-hydrate in water to make 100 mL.

***o*-Phthalaldehyde** $C_8H_6O_2$ [Special class]

***o*-Phthalaldehyde solution** Dissolve 12 mL of ethanol (99.5) in 0.8 g of *o*-phthalaldehyde solution, then add 1.0 g of Brij35 and 2.0 mL of 2-mercaptoethanol, and then dilute to 1000 mL with boric acid and sodium hydroxide buffer solution at pH 10.0.(sodium chondroitin sulfate).

***o*-Phthalaldehyde solution** Weigh accurately 1.0 g of o-phthalaldehyde, dissolve in 5 mL of methanol, add 95 mL of 0.4 mol/L boric acid previously adjusted to pH 10.4 with 4 mol/L potassium hydroxide, and add 2 mL of thioglycolic acid. Adjust pH to 10.4 with 4 mol/L hydroxide solution. (Econazole nitrate,·Triamcinolone acetonide·and Gentamicin Sulfate Cream).

Phthalazine $C_8H_6N_2$ Phthalazine C is a yellow crystal.

Phthalein Purple $C_{32}H_{32}N_2O_{12} \cdot \chi H_2O$ Phthalein Purple is a yellowish white to brown powder, soluble in ethanol and practically insoluble in water.

Sensitivity test: Dissolve 10 mg of Phthalein Purple in 1 mL of ammonia water (28), and add water to make 100 mL. To 5 mL of this solution, add 95 mL of water, 4 mL of ammonia water (28), 50 mL of ethanol, and 0.1 mL of diluted barium chloride TS (1 in 5); the resulting solution exhibits a blue-purple. To this solution, add 0.15 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate TS; the resulting solution is colorless.

Phthalic Acid $C_8H_6O_4$ Phthalic Acid is a colorless to white crystalline powder. It is soluble in ethanol (95) or methanol, slightly soluble in water, and practically insoluble in chloroform.

Melting point: 205 to 209 ℃ (with decomposition).

Content: NLT 98%

Assay: Weigh accurately about 2.8 g of phthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide VS and 25 mL of water, and dissolve by heating on a hot plate. After cooling, add 5 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS. Perform a blank test in the same manner and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 83.06 mg of $C_8H_6O_4$

Phthalic Acid, anhydrous $C_6H_4(CO)_2O$ [Special class]

Picric acid See 2,4,6-trinitrophenol.

Picric acid TS See 2,4,6-trinitrophenol TS.

Picric acid TS, alkaline See 2,4,6-trinitrophenol TS, alkaline.

Picric acid-ethanol TS See 2,4,6-trinitrophenol ethanol TS.

Piperidine hydrochloride $C_5H_{11}N \cdot HCl$ A white, crystalline powder, soluble in water or ethanol. The pH of an aqueous solution of Piperidine Hydrochloride (1 in 20) is 3.0 to 5.0.

Melting point: 240 to 245 ℃

Purity *Clarity and color of solution*: Dissolve 1.0 g of piperidine hydrochloride in 20 mL of water; the solution is clear and colorless.

Residue on ignition: NMT 0.10% (1 g).

Content: NLT 99.0%

Assay: Dissolve about 0.25 g of piperidine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 12.161 mg of $C_5H_{11}N \cdot HCl$

Plasminogen TS Dissolve 1 vial of plasminogen in 25 mL of sterilized phosphate buffer solution (pH 7.4), and store in ice water. Before use, soak in a constant temperature water bath at 37.0°C for 10 to 15 minutes (Streptokinase and Streptodornase)

Plastic, for implantation control Made of polyethylene and

suitable for implantation testing

Platinic acid trichloro ammine See ammine trichloro ammonium platinate.

Platinum-potassium iodide TS

Solution A: Dissolve 6 g of H_2PtCl_6 in 1 mol/L hydrochloric acid to make 100 mL.

Solution B: Dissolve 100 g of potassium iodide in water to make 100 mL.

Spray solution: Mix 5 mL of Solution A and 45 mL of Solution B well, and add water to make 150 mL (doxazosin mesilate).

Poly(cyanopropyl)(7)phenyl(7)methyl(86)siloxane for gas chromatography Prepared for gas chromatography.

Polyalkylene glycol monoether for gas chromatography Prepared for gas chromatography.

Polyamide for thin layer chromatography A polyamide prepared for thin layer chromatography.

Polyamide for thin layer chromatography (with fluorescent indicator) A polyamide for thin layer chromatography containing fluorescent indicator.

Polypropylene glycol for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 400 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 600 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 1500 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 6000 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 15000-diepoxide for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 20 M for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 20000-2-Nitroterephthalate for gas chromatography Prepared for gas chromatography.

Polyethylene glycol esters for gas chromatography Prepared for gas chromatography.

Polyoxyethylene Hydrogenated Castor Oil 60 To prepare Polyoxyethylene Hydrogenated Castor Oil 60, add hydrogen to castor oil to obtain hydrogenated oil, then perform addition polymerization with the hydrogenated oil. As a nonionic surfactant, the average added mole number of ethylene oxide is about 60. A white to pale yellow vaseline-like or lead-like substance having a slightly characteristic odor and a slightly bitter taste. It is very soluble in ethyl acetate or chloroform, freely soluble in ethanol (95), slightly soluble in water, and practically insoluble in ether.

Identification (1) To 0.5 g of Polyoxyethylene Hydrogenated Castor Oil 60, add 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, shake well to mix, add 5 mL of chloroform, shake, mix, and allow to stand; the chloroform layer appears blue.

(2) Add 0.5 g of potassium hydrogen sulfate to 0.2 g of Polyoxyethylene Hydrogenated Castor Oil 60 and heat; a pungent odor similar to acrolein is emitted.

(3) To 0.5 g of Polysorbate 20, add 10 mL of water, shake to mix, and add 5 drops of bromine TS; the color of the test solution does not disappear.

Congealing point: 30 ℃ to 34 ℃.

pH : Add 20 mL of water to 1.0 g of Polyoxyethylene Hydrogenated Castor Oil 60, and dissolve by heating. The pH of the solution is 3.6 to 6.0.

Acid value: NLT 1.0.

Saponification value: 41 to 51.

Hydroxyl value: 39 to 49

Purity (1) *Clarity and color of solution:* Dissolve 1.0 g of Polyoxyethylene Hydrogenated Castor Oil 60 in 20 mL of ethanol; the resulting solution is clear and colorless.

(2) *Heavy metals:* Proceed with 1.0 g of Polyoxyethylene Hydrogenated Castor Oil 60 according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of lead standard solution (NMT 20 ppm).

(3) *Arsenic:* Proceed with 1.0 g of Polyoxyethylene Hydrogenated Castor Oil 60 according to Method 3 and perform the test (NMT 2 ppm).

Water: NMT 2.0% (1 g).

Residue on ignition: NMT 0.10% (1 g).

Packaging and storage: Preserve in tight containers.

Polysorbate 20 Chiefly consists of, addition polymer of sorbitan monolaurate and ethylene oxide. A pale yellow to yellow liquid, having a faint, characteristic odor.

Identification (1) To 0.5 g of Polysorbate 20, add 10 mL of water and 10 mL of sodium hydroxide TS, boil for 5 minutes, and acidify with dilute hydrochloric acid; an oily fraction is separated.

(2) To 0.5 g of Polysorbate 20, add 10 mL of water, shake to mix, and add 5 drops of bromine TS; the red color of the test solution does not disappear.

(3) Saponify 5 g of Polysorbate 20 as directed under the Saponification value, and evaporate ethanol completely. Dissolve the residue in 50 mL of water, acidity with hydrochloric acid (methyl orange), and extract twice with 30 mL each of ether. Combine the ether layer, wash with 20 mL each of water until the washings become neutral, and evaporate the ether on a water bath; the acid value of the residue is 275 to 285. Use 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS for saponification.

Acid value: NMT 4.0.

Saponification value: 43 to 55.

Loss on drying: NMT 3.0% (5 g, 105℃, 1 hour).

Residue on ignition: Weigh accurately 3 g of Polysorbate 20, heat gently at first, and ignite gradually (800 to 1200 °C) until the residue is completely incinerated. If any carbonized substance remains, extract with hot water, filter through a sheet of filter paper for quantitative analysis (5C), and ignite the residue with the filter paper until it turns glowing red. If any carbonized substance still remains, add 15 mL of ether, crush the carbonized substance with a glass rod, burn the ethanol, and ignite carefully. Cool in a desiccator (silica gel), and weigh the residue accurately; starch content is NMT 1.0%.

Polysorbate 80 [See monograph, Part II].

Polystyrene Anion Exchange Resin (polystyrene quarternary ammonium ion-exchange resin) Polystyrene Anion Exchange Resin is a wet resin-like particle containing quaternary ammonium series that can be substituted for an anion. These particles are insoluble and have a slightly amine-like odor. Before use, soak in 10 mL of sodium hydroxide solution (1 in 20) per each mL of resin, then wash until the pH of the last washings is NMT 10.

Polyvinyl alcohol $(-CH_2CHOH-)n$

Polyvinyl alcohol I Colorless to white, or pale yellow granules or powder. It is odorless, or has a faint odor of acetic acid, and is tasteless. Practically insoluble in ethanol (95) or ether. To Polyvinyl Alcohol I, add water, and heat; a clear, viscous solution is obtained. Polyvinyl Alcohol I is hygroscopic.

Viscosity: 25.0 to 31.0 mm^2/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a condenser for 2 hours while stirring. After cooling, add water to make 100.0 mL and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1 ℃ as directed in Method 1 under the Viscosity.

pH: Dissolve 1.0 g of Polyvinyl Alcohol I in 25 mL of water; the pH of the solution is 5.0 to 8.0.

Purity, clarity and color of solution: To 1.0 g of Polyvinyl Alcohol I, add 20 mL of water, disperse by stirring well, warm at 60 °C to 80 ℃ for 2 hours, and cool; the solution is colorless and clear.

Saponification value: 98.0 to 99.0 mol% Weigh accurately 3.0 g of polyvinyl alcohol I, previously dried, place it in a flask, add 100 mL of water, and heat on a water bath to dissolve. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then, add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake well, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner and make any necessary correction. However, when the volume of 0.1 mol/L sodium hydroxide VS consumed in the test is 25mL or more, use about 2.0 g of the sample.

a : Amount (mL) of 0.1 mol/L sodium hydroxide solution consumed

b : Amount (mL) of 0.1 mol/L sodium hydroxide solution consumed in the blank test

f: Factor of 0.1 mol/L sodium hydroxide VS

Polyvinyl alcohol II Colorless to white or pale yellow granules or powder. It is odorless, or has a faint odor of acetic acid, and is tasteless. Practically insoluble in ethanol (95) or ether. To Polyvinyl Alcohol II, add water, and heat; a clear, viscous solution is obtained. Polyvinyl Alcohol II is hygroscopic.

Viscosity: 4.6 to 5.4 mm^2/s Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL of water, allow to stand for 30 minutes, and shake to dissolve for 2 hours at 60 to 80 ℃. After cooling, add water to make 100.0 g and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1 ℃ as directed in Method 1 under the Viscosity.

pH: Dissolve 1.0 g of Polyvinyl Alcohol II in 25 mL of water; the pH of the solution is 5.0 to 8.0.

Purity *Clarity and color of solution*: To 1.0 g of Polyvinyl Alcohol II, add 20 mL of water, disperse by mixing well, warm 60 °C to 80 ℃ for 2 hours, and cool: the solution is colorless and clear.

Saponification value: 86.5 to 89.5 mol%. Dry Polyvinyl Alcohol II, weigh 2.0 g accurately, place it in a Erlenmeyer flask, add 100 mL of water, and heat on a water bath to dissolve. After cooling, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then, add exactly 30 mL of 0.25 mol/L sulfuric acid VS, shake well, and titrate with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner and make any necessary correction.

a : Amount (mL) of 0.5 mol/L sodium hydroxide solution consumed

b : Amount (mL) of 0.5 mol/L sodium hydroxide solution consumed in the blank test

f : Factor of 0.5 mol/L sodium hydroxide VS

Polyvinyl alcohol TS Suspend 20 g of polyvinyl alcohol (average degree of polymerization: 1725 ± 25, content: 95.0 ± 1.0%) in about 800 mL of water, and shake occasionally to mix. Heat at 75 - 80 ℃ for about 1 hour, cool, filter if necessary, and add water to make 1 L (Newlase).

Polyvinyl alcohol TS Weigh exactly 0.50 g of polyvinyl alcohol, and add water to make exactly 100 mL.

Porous acrylonitrile-divinylbenzene copolymer for gas chromatography (0.06 to 0.08 µm pore, 100 to 200 m^2/g) Prepared for gas chromatography.

Porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average diameter 0.0075 µm, 500 to 600 m^2/g) Prepared for gas chromatography.

Porous polymer beads for gas chromatography Prepared for gas chromatography.

Porous silica for liquid chromatography Prepared for liquid chromatography.

Porous silica gel for liquid chromatography Prepared for liquid chromatography.

Potassium acetate CH_3COOK [Special class]

Potassium acetate TS Dissolve 10 g of potassium acetate in water to make 100 mL (1 mol/L).

Potassium bicarbonate $KHCO_3$ [Potassium bicarbonate (Potassium bicarbonate), Special Class]

Potassium Bismuth Iodide TS Dissolve 100 g of tartaric acid in 400 mL of water, add 8.5 g of bismuth subnitrate (pharmacopoeia), and shake the mixture for 1 hour to dissolve. Then, add 2000 mL of 40% potassium iodide solution, shake vigorously for mixing, allow to stand for 24 hours, and then filter (Solution A). Separately, dissolve 100 g of tartaric acid in 500 mL of water (Solution B). To Solution B, add 50 mL of Solution A and mix (trimethoprim).

Potassium bromate $KBrO_3$ [Special class].

Potassium bromide KBr [Special class].

Potassium bromide, for Mid-infrared Spectroscopy Crush potassium bromide single crystal or potassium bromide, collect the powder passed through a No. 200 (75 μm) sieve, and dry at 120 °C for 10 hours or at 500 °C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum under the Mid-infrared Spectroscopy; it does not exhibit any abnormal absorption.

Potassium carbonate K_2CO_3 [See monograph, Part II]

Potassium carbonate-sodium carbonate TS Dissolve 1.7 g of sodium hydroxide and 1.3 g of sodium acetate trihydrate with water to make 100 mL.

Potassium carbonate, anhydrous K_2CO_3 [Potassium carbonate (anhydrous), Special Class]

Potassium chlorate $KClO_3$ [Special Class]

Potassium chloride KCl [Special Class]

Potassium chloride TS, 0.2 mol/L Dissolve 14.9 g of potassium chloride in water to make 1000 mL. Prepare before use.

Potassium chloride TS, acidic Dissolve 250 g of potassium chloride in water to make 1000 mL, and to this solution, add 8.5 mL of hydrochloric acid.

Potassium chloride-hydrochloric acid buffer solution To 250 mL of a solution of potassium chloride (3 in 20) and 53 mL of 2 mol/L hydrochloric acid TS, add water to make 1000 mL.

Potassium chloride-hydrochloric acid buffer solution, pH 2.0 Add 88 mL of 0.2 mol/L potassium chloride TS to 10 mL of 0.2 mol/L hydrochloric acid, adjust the pH to 2.0 with 0.2 mol/L hydrochloric acid, and then add water to make 200 mL.

Potassium chloride-hydrochloric acid buffer solution, pH 2.2 Add 67 mL of 0.1 mol/L hydrochloric acid to 500 mL of 0.1 mol/L potassium chloride TS 500 and add water to make 1 L.

Potassium chromate K_2CrO_4 [Special Class]

Potassium chromate TS Dissolve 10 g of potassium chromate with water to make 100 mL.

Potassium cyanide KCN [Special class].

Potassium cyanide solution Dissolve 50 g of potassium cyanide in water to make 100 mL. Extract with 20 mL of extracting dithizone solution until the extract turns green to remove lead, and add chloroform and shake to mix to remove the remaining dithizone. Add water to this solution to contain 10 g of potassium cyanide in 100 mL.

Potassium cyanide TS Dissolve 1 g of potassium cyanide in water to make 10 mL. Prepare before use.

Potassium dichromate $K_2Cr_2O_7$ [Special Class]

Potassium dichromate See potassium dichromate.

Potassium dichromate (standard reagent) $K_2Cr_2O_7$ [Reference material for volumetric analysis]

Potassium dichromate (standard reagent) See potassium dichromate (standard reagent).

Potassium dichromate TS Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

Potassium dichromate TS See potassium dichromate TS.

Potassium dichromate-sulfuric acid solution A solution prepared by dissolving 3 g of potassium dichromate in 20 mL of water and mixing with 10 mL of sulfuric acid (thioctic acid amide).

Potassium dichromate-sulfuric acid TS Dissolve 0.5 g of potassium dichromate in dilute sulfuric acid (1 in 5) to make 100 mL.

Potassium dichromate-sulfuric acid TS See potassium dichromate-sulfuric acid TS.

Potassium dihydrogen phosphate KH_2PO_4 [Special Class]

Potassium dihydrogen phosphate buffer solution, 0.05 mol/L, pH 6.8 Dissolve 6.805 g of potassium dihydrogen phosphate and 0.88 g of sodium hydroxide in water, adjust the pH to 6.8 with 1 mol/L sodium hydroxide solution, and add water to make exactly 1000 mL.

Potassium dihydrogen phosphate for pH measurement See potassium dihydrogen phosphate, for pH measurement.

Potassium dihydrogen phosphate solution, pH 3.0 Dissolve 6.8 g of potassium dihydrogen phosphate and 2.2 g of sodium heptane sulfonate in water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1 L.

0.2 mol/L Potassium dihydrogen phosphate TS for buffer solution See potassium dihydrogen phosphate TS, 0.2 mol/L, for buffer solution.

Potassium dihydrogen phosphate TS, 0.02 mol/L Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.05 mol/L Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.05 mol/L, pH 3.0 Add phosphoric acid to 0.05 mol/L potassium dihydrogen phosphate TS to adjust the pH to 3.0.

Potassium dihydrogen phosphate TS, 0.05 mol/L, pH 4.7 Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 4.7 exactly with dilute sodium hydroxide TS, and add water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.05 mol/L, pH 7.0 Add trimethylamine to 0.05 mol/L potassium dihydrogen phosphate TS to adjust the pH to 7.0.

Potassium dihydrogen phosphate TS, 0.2 mol/L Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.2 mol/L, for buffer solution Dissolve 27.218 g of potassium dihydrogen phosphate for pH measurement in water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.25 mol/L pH 3.0 Dissolve potassium dihydrogen phosphate 4.491 g in about 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

Potassium dihydrogen phosphate TS, 0.33 mol/L Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium dihydrogen phosphate TS, 2 mol/L Dissolve 312.02 g of potassium dihydrogen phosphate in water to make 1000 mL.

Potassium dihydrogen phosphate, 0.02 mol/L, pH 3.5 Add phosphoric acid to 0.02 mol/L potassium dihydrogen phosphate TS to adjust the pH to 3.5 (domperidone tablet).

Potassium dihydrogen phosphate, for pH measurement KH_2PO_4 [Monopotassium phosphate, for pH measurement]

Potassium disulfate $K_2S_2O_7$ [Special Class] Preserve in a stoppered container in a cold and dark place away from fire.

Potassium ferricyanide See potassium hexacyanoferrate(III).

Potassium ferricyanide TS Dissolve 1 g of Potassium ferricyanide in water to make 100 mL. Take 4 mL of this solution, and add sodium hydroxide solution (1 in 7) to make 100 mL. Prepare before use. (Fursultiamine)

Potassium ferricyanide TS See potassium hexacyanoferrate(III) TS.

Potassium ferricyanide TS, alkaline See potassium hexacyanoferrate(III) TS, alkaline.

Potassium ferrocyanide See potassium hexacyanoferrate(II) trihydrate.

Potassium ferrocyanide TS See potassium hexacyanoferrate(II) TS.

Potassium guaiacolsulfonate $C_7H_7KO_5S$ [See monograph, Part I]

Potassium hexacyanoferrate(II) trihydrate $K_4Fe(CN)_6 \cdot 3H_2O$ [Special class]

Potassium hexacyanoferrate(II) TS Dissolve 1 g of potassium hexacyanoferrate(II) trihydrate in water to make 10 mL. Prepare before use (0.25 mol/L).

Potassium hexacyanoferrate(III) $K_3Fe(CN)_6$ [Special class]

Potassium hexacyanoferrate(III) TS Dissolve 1 g of potassium hexacyanoferrate(III) in water to make 10 mL. Prepare before use (0.3 mol/L).

Potassium hexacyanoferrate(III) TS Dissolve 1 g of potassium hexacyanoferrate(III) in water to make 100 mL. Take 4 mL of this solution, and add sodium hydroxide solution (1 in 7) to make 100 mL. Prepare Potassium Hexacyanoferrate(III) TS immediately prior to use (fursultiarmine).

Potassium hexacyanoferrate(III) TS, 3% Dissolve 3 g of potassium hexacyanoferrate(III) in 60 mL of water and 40 mL of 96% ethanol.

Potassium hexacyanoferrate(III) TS, alkaline Dissolve 1.65 g of potassium hexacyanoferrate(III) and 10.6 g of anhydrous sodium carbonate in water to make 1000 mL. Preserve in light-resistant containers.

Potassium hexahydroxoantimanate(V) $KSb(OH)_6$ [First class]

Potassium hexahydroxoantimanate(V) TS Add 100 mL of water to 2 g of potassium hexahydroxoantimanate(V), boil for about 5 minutes, and quickly cool. Add 10 mL of potassium hydroxide solution (3 in 20) to this solution, allow to stand for a day, and filter.

Potassium hydrogen phthalate $C_6H_4(COOK)(COOH)$ [Special class]

Potassium hydrogen phthalate (standard reagent) $C_6H_4(COOK)(COOH)$ [standard substance for volumetric analysis]

Potassium hydrogen phthalate buffer solution, pH 3.0 Add 40.6 mL of 0.1 mol/L hydrochloric acid to 100 mL of 0.1 mol/L potassium hydrogen phthalate TS, and add water to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 3.5 To a mixture of 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97 mL of 0.2 mol/L hydrochloric acid, add water to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 3.6 Add 1 mol/L hydrochloric acid to 4% potassium hydrogen phthalate solution to adjust the pH to 3.6.

Potassium hydrogen phthalate buffer solution, pH 4.4 Add water to 50 mL of 0.2 mol/L potassium hydrogen phthalate solution and 7.5 mL of 2 mol/L sodium hydroxide to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 4.5 Dissolve 60 g of potassium hydrogen phthalate in water, add 80 mL of sodium hydroxide TS with water to make 1000 mL.

Potassium hydrogen phthalate buffer solution, pH 4.6 Add 0.2 mol/L sodium hydroxide TS to 50 mL of 0.2 mol/L potassium hydrogen phthalate TS, adjust the pH to 4.6, and add water to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 4.6 To a mixture of 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0 mL of 0.2 mol/L sodium hydroxide solution, add water to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 5.6 To a mixture of 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7 mL of 0.2 mol/L sodium hydroxide, add water to make 200 mL.

Potassium hydrogen phthalate for pH measurement See potassium hydrogen phthalate, for pH measurement.

0.2 mol/L Potassium hydrogen phthalate TS for buffer solution See potassium hydrogen phthalate TS, 0.2 mol/L, for buffer solution.

Potassium hydrogen phthalate TS, 0.2 mol/L, for buffer solution Dissolve 40.843 g of potassium hydrogen phthalate for pH measurement in water to make 1000 mL.

Potassium hydrogen phthalate, for pH measurement $C_6H_4(COOK)(COOH)$ [for pH measurement]

Potassium hydrogen sulfate $KHSO_4$ [Special class]

Potassium hydroxide KOH [Special class]

20% Potassium hydroxide solution Dissolve 20 g of potassium hydroxide in 100 mL of 80% low carbonyl methanol solution (nalbuphine Hydrochloride).

Potassium hydroxide TS Dissolve 6.5 g of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

Potassium hydroxide TS, 0.02 mol/L Dilute 2 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

Potassium hydroxide TS, 0.05 mol/L Dilute 5 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

Potassium hydroxide TS, 8 mol/L Dissolve 52 g of potassium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

Potassium hydroxide-ethanol TS Dissolve 10 g of potassium hydroxide in ethanol to make 100 mL. Prepare before use.

Potassium hydroxide-ethanol TS, 0.1 mol/L To 1 mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5 mL. Prepare before use.

Potassium hydroxide-ethanol TS, 0.5 mol/L Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol to make 1000 mL. Preserve in tightly stoppered bottles.

Potassium hydroxide-ethanol TS, dilute See potassium hydroxide-ethanol TS, 0.5 mol/L.

Potassium hydroxide-glycerin TS Add 17.5 g of potassium hydroxide in glycerin, dissolve by warming to make 100 mL (linoleic acid).

Potassium hydroxide-methanol TS, 0.1 mol/L Dissolve 6.8 g of potassium hydroxide in 4 mL of water and dilute with methanol to make 1000 mL.

Potassium iodate KIO_3 [Special Class]

Potassium iodate (standard reagent) KIO_3 [Reference material for volumetric analysis]

Potassium iodate starch paper Impregnate filter paper with a mixture of equivalent volumes of a solution of potassium iodate (1 in 20) and freshly prepared starch TS, and dry in a clean room. Preserve in a glass-stoppered bottle, protected from light and moisture.

Potassium iodide KI [Special Class]

Potassium iodide solution, 30% Dissolve 30 g of potassium iodide in water to make 100 mL. Prepare before use and then store away from light (diastase and protease 500).

Potassium iodide starch paper Impregnate the filter paper with freshly prepared potassium iodide-starch TS, and dry in a clean room. Preserve in a glass-stoppered bottle and store away from light and moisture.

Potassium iodide starch TS Dissolve 0.5 g of potassium iodide in 100 mL of freshly prepared starch TS. Prepare before use.

Potassium iodide TS Dissolve 16.5 g of potassium iodide in water to make 100 mL. Store away from light. Prepare before use (1 mol/L).

Potassium iodide TS, concentrated Dissolve 30 g of potassium iodide in 70 mL of water. Prepare before use.

Packaging and storage: Store away from light.

Potassium iodide-bismuth TS Dissolve 100 g of tartaric acid in 400 mL of water, add 8.5 g of bismuth subnitrate, and shake the mixture for 1 hour to dissolve. Then, add 2000 mL of 40% potassium iodide solution, shake vigorously for mixing, allow to stand for 24 hours, and then filter (Solution A). Separately, dissolve 100 g of tartaric acid in 500 mL of water (Solution B). To Solution B, add 50 mL of Solution A and mix.

Potassium iodide-platinic chloride TS See hexachloroplatinic(IV) acid-potassium iodide TS.

Potassium iodide-zinc sulfate TS Dissolve 5 g of potassium iodide, 10 g of zinc sulfate heptahydrate, and 50 g of sodium chloride in water to make 200 mL.

Potassium methanesulfonate CH_3SO_3K White crystals or crystalline powder.

Purity *Clarity and color of solution*: Dissolve 1.0 g of potassium methanesulfonate in 20 mL of water; the solution is clear and colorless.

Content: NLT 98.0%.

Assay: Weigh accurately about 0.1 g of potassium methanesulfonate, dissolve in 10 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.42 mg of CH_3SO_3K

Potassium monohydrogen phosphate See dibasic potassium phosphate.

Potassium monohydrogen phosphate TS, 1 mol/L, for buffer solution See dibasic potassium phosphate TS, 1 mol/L, for buffer solution.

potassium monohydrogen phosphate-citric acid buffer solution, pH 5.3 See dibasic potassium phosphate, pH 5.3.

Potassium naphthoquinonesulfonate See potassium 1,2-naphthoquinone-4-sulfonate.

Potassium naphthoquinonesulfonate TS See potassium 1,2-naphthoquiaone-4-sulfate TS.

Potassium nitrate KNO_3 [Special Class]

Potassium nitrite KNO_2 [Special grade]

Potassium nitrite TS Dissolve 10 g of potassium nitrite in water to make 100 mL. Prepare before use.

Potassium perchlorate $KClO_4$ [Special class]

Potassium periodate KIO_4 [*m*-Potassium periodate, Special class]

Potassium periodate TS To 2.8 g of potassium periodate, add 200 mL of water, add 20 mL of sulfuric acid dropwise while shaking, cool, and then add water to make 1000 mL.

Potassium permanganate KMnO4 [Special class]

Potassium permanganate TS Dissolve 3.3 g of potassium permanganate in water to make 1000 mL (0.02 mol/L).

Potassium permanganate TS, acidic To 100 mL of potassium permanganate TS, add 0.3 mL of sulfuric acid.

Potassium peroxydisulfate $K_2S_2O_8$ [Special class]

Potassium persulfate See potassium peroxydisulfate.

Potassium phosphate buffer solution, pH 6.0 Dissolve 0.65 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 6.0 with potassium carbonate solution (1 in 1000).

Potassium pyroantimonate See potassium hexahydroxoantimonate (V).

Potassium pyroantimonste TS See potassium hexahydroxoantimonate(V) TS.

Potassium pyrophosphate $K_4O_7P_2$ A white, crystalline powder, very soluble in water.

Melting point: 1109℃

Potassium pyrosulfate See potassium disulfate.

Potassium sodium tartrate See potassium sodium tartrate tetrahydrate.

Potassium sodium tartrate See potassium sodium tartrate tetrahydrate.

Potassium sodium tartrate tetrahydrate $KNaC_4H_4O_6 \cdot 4H_2O$ [Potassium sodium tartrate tetrahydrate (rochelle salt, seignette salt), Special Class]

Potassium sulfate K_2SO_4 [Special class]

Potassium sulfate aluminum See aluminium potassium sulfate dodecahydrate.

Potassium sulfate solution Dissolve 1.814 g of potassium sulfate in water to make 1000 mL. To 10 mL of the solution, add 30 mL of ethanol, and then add water to make 100 mL (calcium fluoride).

Potassium sulfate TS Dissolve 1 g of potassium sulfate in water to make 100 mL.

Potassium tellurite K_2TeO_3 White powder or small mass obtained from dissolution of the mixture of mol equivalents of the tellurium dioxide and potassium carbonate in the carbon dioxide air flow. It is soluble in water.

Content: NLT 90.0%

Assay: Weigh accurately about 1.0 g of potassium tellurite, dissolve in 100 mL of water, add 5 mL of dilute acetic acid (1 in 3), and boil. Cool it, and filter by suction with a glass filter [constant weight obtained after drying for 1 hour at 105 ± 2 ℃ (*b* (g))]. Wash the filtrate with water, dry it with a glass filter at 110 ℃ for 3 hours, and then measure the mass *a*(g).

$$\text{Content (\%) of potassium tellurite} = \frac{(a-b)\times 1.5902}{S}\times 100$$

S: Amount of sample taken

Potassium tetraphenylborate TS To 50 mL of a solution of potassium hydrogen phthalate (1 in 500), add 1 mL of acetic acid (31). To this solution, add 20 mL of sodium tetraphenylborate solution (7 in 1000), shake well to mix, allow to stand for 1 hour, collect the precipitates formed and wash with water. Take about 1/3 of the precipitates, add 100 mL of water, shake to mix at about 50 ℃ and warm the mixture for 5 minutes. Then, cool promptly, allow to stand for 2 hours with occasional shaking at ordinary temperature, and then filter. Discard the first 30 mL of the filtrate.

Potassium thiocyanate KSCN [Special Class]

Potassium thiocyanate TS See potassium thiocyanate.

Potassium thiocyanate TS To 1 g of potassium thiocyanate, add water to dissolve and make 10 mL.

Potassium trihydrogen dioxalate dihydrate for pH measurement See potassium trihydrogen dioxalate dihydrate, for pH measurement.

Potassium trihydrogen dioxalate dihydrate, for pH measurement $KH_3(C_2O_4)_2 \cdot 2H_2O$ [for pH measurement]

Potato starch solution TS for starch digestion test See potato starch TS, for starch digestibility test.

Potato starch solution, 1% Weigh accurately 1 g of potato starch, previously dried at 105 ℃ for 2 hours, dissolve in 20 mL of water, and slowly add 5.0 mL of 2 mol/L sodium hydroxide solution to form a gel. Heat on a water bath for 30 minutes, add 25 mL of water, and cool. Then add 2 mol/L hydrochloric acid to neutralize, and add 10 mL of phosphate buffer solution, pH 7.0 to make 100 mL (pancreatic digestive enzyme TA).

Potato starch solution, 1%, pH 5.0 Weigh accurately about 1 g of potato starch, dry at 105 ℃ for 2 hours, then measure the weight loss. Weigh accurately an amount equivalent to 1.0 g of potato starch, previously dried, add 20 mL of water, and shake to mix. Slowly add 5.0 mL of 2 mol/L sodium hydroxide TS to form a gel and heat on a water bath for 3 minutes while shaking vigorously, and add 25 mL of water. After cooling, neutralize with 2 mol/L hydrochloric acid TS, and add 10 mL of acetic acid-sodium acetate buffer solution, pH 5.0 and add water to make 100 mL. Prepare before use (diastase·protease N1).

Potato starch TS Prepare as directed under starch TS with 1 g of potato starch.

Potato starch TS, 2% Weigh accurately 2.0 g of potato starch, mix with a small amount of water, slowly put it in 50 mL of boiling water, and continue boiling for 5 minutes. After cooling, add 10 mL of Macklebane buffer solution, pH 5.0 and water to make 100 mL.

Potato starch TS, for starch digestion test Weigh accurately about 1 g of potato starch, dry at 105 ℃ for 2 hours, and measure the loss. Weigh accurately an amount equivalent to about 1.000 g of potato starch, previously dried, transfer into an Erlenmeyer flask, add 20 mL of water, and make it pasty by slowly adding 5 mL of a solution of sodium hydroxide (2 in 25) while shaking well. Then, heat for 3 minutes on a water bath while shaking, then add 25 mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid, add 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0, and add water to make exactly 100 mL. Prepare before use.

Precipitation TS Make a solution containing 0.11 mol/L trichloroacetic acid, 0.22 mol/L sodium acetate and 0.33 mol/L acetic acid (bromelain).

Prednisolone $C_{21}H_{28}O_5$ [See monograph, Part I]

Prednisone $C_{21}H_{26}O_5$ A white, crystalline powder. Slightly soluble in methanol, in ethanol (95) and in chloroform, and very slightly soluble in water.

Specific optical rotation $[\alpha]_D^{20}$: +167 to +175° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Loss on drying: NMT 1.0% (1 g, 105 ℃, 3 hours).

Content: 96.0 to 104.0%

Assay: Weigh accurately about 20 mg of prednisone, previously dried, and dissolve in methanol to make exactly 100 mL. Dilute 5 mL of this solution to make exactly 100 mL. Perform the test with this solution as directed under the Ultraviolet-visible Spectroscopy, and determine the absorbance, *A*, at the wavelength of about 238 nm with maximum absorption.

Procainamide hydrochloride [See monograph, Part I].

Procaine hydrochloride $C_{13}H_{20}N_2O_2{\cdot}HCl$ [See monograph, Part I]

Procaterol Hydrochloride $C_{16}H_{22}N_2O_3 \cdot HCl \cdot 1/2H_2O$ [See monograph, Part I on Procaterol Hydrochloride Hydrate]

Progesterone $C_{21}H_{30}O_2$ [See monograph, Part I]

L-Proline $C_5H_9NO_2$ [Special class]

2-Propanol for liquid chromatography $(CH_3)_2CHOH$ A clear, colorless and volatile liquid, having a characteristic odor. Miscible with water, ethanol or ether.

Boiling point: About 82 ℃

Refractive index n_D^{20}: 1.376 to 1.378

Specific gravity d_{20}^{20}: 0.785 to 0.788

Purity (1) *Ultraviolet absorbing substances:* Perform the test with 2-Propanol for Liquid Chromatography as directed under the Ultraviolet-visible Spectroscopy, using water as the blank; the absorbance at 230 nm is NMT 0.2; at 250 am, NMT 0.03; and at 280 nm to 400 nm, NMT 0.01.

(2) *Peroxide:* To 20 g of 2-Propanol for Liquid Chromatography, add a solution previously prepared by adding 25 mL of potassium iodide solution (1 in 10) to 100 mL of water and 25 mL of dilute sulfuric acid. Stopper it, shake to mix, and allow to stand for 15 minutes in a dark place. Titrate it with 0.01 mol/L sodium thiosulfate solution (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (NMT 0.0005%).

1-Propanol $CH_3CH_2CH_2OH$ [Special class]

2-Propanol $(CH_3)_2CHOH$ [Isopropyl Alcohol (Isopropanol), Special class]

***n*-Propanol** See 1-propanol.

Propantheline bromide $C_{23}H_{30}BrNO_3$ [See monograph, Part I]

Propionic acid CH_3CH_2COOH Propionic Acid is a colorless liquid.

Purity *Clarity and color of solution*: Dissolve 1 g of Propionic Acid in 20 mL of ethanol (95); the solution is clear and colorless.

Specific gravity d_{20}^{20}: 0.998 to 1.004

Distilling range: 139 to 143 ℃, NLT 95 vol%.

Propyl benzoate $C_6H_5COOC_3H_7$ A clear, colorless liquid, having a characteristic odor.

Refractive index n_D^{20}: 1.498 to 1.503

Specific gravity d_{20}^{20}: 1.022 to 1.027

Propyl p-hydroxybenzoate $HOC_6H_4COOCH_2CH_2CH_3$ [See monograph, Part II]

Propylamine, iso $(CH_3)_2CHNH_2$ A colorless liquid, having a characteristic, amine-like odor. Miscible with water, ethanol or ether.

Specific gravity d_{20}^{20}: 0.685 to 0.690

Refractive index n_D^{20}: 1.374 to 1.376

Distilling range: 31 to 33 ℃, NLT 95 vol%

Propylene glycol $CH_3CH(OH)CH_2OH$ [Special class]

Propylene carbonate $C_4H_6O_3$ Colorless liquid.

Melting point: 240 ℃ to 242 ℃.

Water: NMT 1 mg per g.

Propylether, iso $(CH_3)_2CHOCH(CH_3)_2$ A clear, colorless liquid, having a characteristic odor. It is not miscible with water.

Specific gravity d_4^{20}: 0.723 to 0.725

Refractive index n_D^{20}: 1.368 to 1.369

Prostaglandin A_1 $C_{20}H_{32}O_4$ White crystals or crystalline powder. Very soluble in ethanol (95) or ethyl acetate, and practically insoluble in water.

Purity *Related substances*: Dissolve 5 mg of Prostaglandin A_1 in 10 mL of ethanol (95), and use this solution as the test solution. Pipet 3 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the test solution and standard solution as directed under the Liquid Chromatography under the following conditions. Determine areas of all peaks of both solutions by the automatic integration method: the total area of the peaks other than the peak of prostaglandin A_1 from the test solution is not larger than the peak area of prostaglandin A_1 from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, flow rate and selection of column: Proceed as directed in the operating conditions in the Assay of Alprostadil Alfadex.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of prostaglandin A_1 obtained from10 μL of the standard solution composes 5 to 10% of the full scale.

Time span of measurement: About twice the retention time of prostaglandin A_1 after the solvent peak.

Purified hydrochloric acid See hydrochloric acid, purified.

Purified methanol See Methanol, purified.

Purified sulfuric acid See sulfuric acid, purified.

Purified water [See monograph, Part II]

Purified water, for ammonium test Carefully add 4.5 mL of sulfuric acid to 1500 mL of purified water, distill it with hard glass distiller. Sufficiently discard the initial distillate, and use the subsequent distillate as the ammonium-free purified water.

Purity: Mix 40 mL of this drug with 6.0 mL of phenol-sodium pentacyanonitrosylferrate(III) TS. Then, add 4.0 mL of sodium hypochlorite-sodium hydroxide TS for mixing, and allow to stand for 60 minutes. Perform the test with this solution as directed under the Ultraviolet-visible Spectroscopy using water as the blank: the absorbance at 640 nm is NMT 0.10.

Pyrazole $C_3H_4N_2$ White to pale yellow crystals or crystalline powder.

Melting point: 67 to 71 ℃

Pyridine C_5H_5N [Special class]

Pyridine for Karl Fischer method See Water Determination.

Pyridine for Karl Fischer titration See the Water Determination under the General Tests.

Pyridine-acetic acid TS To 20 mL of pyridine, add diluted acetic acid (100) (1 in 25) to make 100 mL. Prepare before use.

Pyridine-pyrazolone TS Dissolve 100 mg of 3-methyl-1-phenyl-5-pyrazolone in 100mL of water by heating at 65 to 70 °C, with thorough shaking, and cool to below 30 °C. Mix this solution with a solution prepared by dissolving 20 mg of bis-(1-phenyl-3- methyl-5-pyrazolone) in 20 mL of pyridine. Prepare before use.

Pyridine, anhydrous C_5H_5N To 100 mL of pyridine, add 10 g of potassium hydroxide, and allow to stand for 24 hours. Decant the supernatant, and distill.

Pyridoxine hydrochloride $C_8H_{11}NO_3 \cdot HCl$ [See monograph, Part I]

1-(4-Pyridyl) pyridinium chloride hydrochloride $C_{10}H_9ClN_2 \cdot HCl$ A white to yellowish white crystalline powder. It is very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in ether.

Melting point: 154 to 156 ℃

3-(2-Pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid sodium $C_{16}H_8N_4Na_2O_8S_2$ [First class]

1-(2-Pyridylazo)-2-naphthol $C_{15}H_{11}N_3O$ An orange-yellow or orange-red powder.

Melting point: 137 to 140 ℃

Purity *Clarity and color of solution*: Dissolve 25 mg of 1-(2-Pyridylazo)-2-Naphthol in 100 mL of methanol; the resulting solution is orange-yellow and clear.

Sensitivity: To 0.2 mL of the methanol solution (1 in 4000) of 1-(2-Pyridylazo)-2-Naphthol, add 50 mL of water, 30 mL of methanol, and 10 mL of acetic acid-sodium acetate buffer solution (pH 5.5); the resulting solution appears yellow. Add 1 drop of copper(II) chloride dihydrate solution (1 in 600) to this solution; the solution appears reddish-purple. Add 1 drop of diluted 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate TS (1 in 10); it turns yellow again.

Absorbance: Weigh exactly about 25 mg of 1-(2-pyridylazo)-2-naphthol, and dissolve in methanol to make exactly 100 mL. Take 2.0 mL of this solution, and add methanol to make exactly 50 mL. Determine the absorbance at a wavelength of 470 nm using methanol as the blank; the absorbance is NLT 0.55.

Residue on ignition: NMT 1.0%.

Pyrogallol $C_6H_3(OH)_3$ [Special class]

Pyrogallol sulfate solution Add 15 mL of sulfuric acid to 0.2 g of pyrogallol, mix well, heat on a water bath for 20 minutes, cool, and add water to make 20 mL (manganese dioxide).

1-Pyroglutamyl glycyl-L-arginine-*p*-nitroanilide hydrochloride $C_{19}H_{26}N_8O_6 \cdot HCl$ A white to pale yellow powder. Freely soluble in water, methanol, or acetic acid (100).

Absorbance $E_{1cm}^{1\%}$ (316 nm): 242 to 268 (2 mg, water, 100 mL)

Specific optical rotation $[\alpha]_D^{25}$: -51 to -56° [0.1 g, dilute acetic acid (100)(1 in 2), 10 mL, 100 mm]

Purity *Related substances*: Dissolve 0.05 g of 1-Pyroglutamyl Glycyl-L-Arginine-*p*-Nitroanilide Hydrochloride in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 20 μL each of the test solution and the standard solution on the thin plate made of silica gel for thin-layer chromatography (with fluorescent agent). Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15 : 12 : 10 : 3) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot obtained from the test solution are not more intense than those from the standard solution

1-pyroglutamyl glycyl-L-arginine-*p*-nitroanilide TS Dissolve 25 mg of 1-pyroglutamyl glycyl-L-arginine-*p*-nitroanilide hydrochloride and 40 mg of D-mannitol in 2 to 3 mL of water, and freeze-dry. Add 16.7 mL of water here and dissolve.

Pyrophosphate buffer solution, 0.05 mol/L, pH 9.0 Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water, add 1 mol/L hydrochloric acid to adjust the pH to 9.0, and add water to make 50 mL. Set the temperature to 22 ± 2 ℃ before use.

Pyrophosphate buffer solution, pH 9.0 Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol, and 40 mg of disodium dihydrogen ethylenediaminetetraacetate dihydrate in 70 mL of water, adjust the pH to exactly 9.0 with citric acid solution (21 in 100), and add water to make 100 mL.

Pyrrole C_4H_5N A clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) or ether, and practically insoluble in water.

Specific gravity d_{20}^{20}: 0.965 to 0.975

Quinaldine red (5-dimethylamino-2-styrylethylquinolinium iodide) Blackish blue powder. Practically insoluble in water. Freely soluble in ethanol.

Melting point: About 260 ℃

Range of color change: pH 1.4 (colorless) to 3.2 (red)

Quinaldine red TS Dissolve quinaldine red 100 mg in acetic acid (100) 100 mL.

Quinhydrone $C_6H_4(OH)_2 \cdot C_6H_4O_2$ Green crystals or crystalline powder.

Melting point: 169 to 172 ℃

Quinoline C_9H_7N [Special Class]

Quinoline TS Mix 50 mL of quinoline with 360 mL of diluted hydrochloric acid (1 in 6), previously warmed, cool the mixture, and if necessary, filter it.

8-Quinolinol C_9H_7NO [Special Class]

R TS Dissolve 4 g of copper(II) sulfate pentahydrate, 24.0 g of sodium carbonate, 16.0 g of sodium bicarbonate, 18.0 g of sodium sulfate and 12 g of potassium sodium tartrate tetrahydrate in water, cover with stopper, and allow to stand for 1 week. Then filter with a glass filter, and store in a brown bottle.

Raney nickel, for catalyst A grayish black powder. An alloy containing 40% to 50% of nickel and 50% to 60% of aluminum.

Raney nickel, for catalyst See raney nickel, for catalyst.

Red blood cell suspension for type A See red blood cell suspension, for type A.

Red blood cell suspension for type B See red blood cell suspension, for type B.

Red litmus paper See litmus paper, red.

Red phosphorus P [First Class].

Reduced iron Fe [Special class]

Reinecke salt See reinecke salt monohydrate.

Reinecke salt monohydrate $NH_4[Cr(NH_3)_2(SCN)_4] \cdot H_2O$ [Special class]

Reinecke salt TS To 0.5 g of Reinecke salt monohydrate, add 20 mL of water, shake frequently to mix for 1 hour, then filter. Use within 48 hours.

Resazurin $C_{12}H_6NNaO_4$ Brownish violet crystalline powder. Dissolve in water; it exhibits a violet color.

Residue on ignition: NMT 28.5% (1 g).

Resorcin See resorcinol.

Resorcin TS See resorcinol TS.

Resorcin-sulfuric acid TS See resorcinol-sulfuric acid TS.

Resorcinol $C_6H_4(OH)_2$ [Special class]

Resorcinol TS Dissolve 0.1 g of resorcinol in 10 mL of hydrochloric acid. Prepare before use.

Resorcinol-sulfuric acid TS Dissolve 0.1 g of resorcinol in 10 mL of diluted sulfuric acid (1 in 10).

Rhodamine B $C_{28}H_{31}ClN_2O_3$ [Tetraethylrhodamine] Green crystals or reddish puple powder. Very soluble in water. Its solution exhibits a bluish red color and is strongly fluorescent when diluted. Very soluble in alcohol, and slightly soluble in dilute acids and alkali solutions. In a strong acid solution, it forms a complex compound with antimony that is soluble in isopropyl ether.

Clarity and color of solution: Its solution (1 in 200) is clear.

Residue on ignition: Ignite 1 g of rhodamine B with 1 mL of sulfuric acid; the amount of the residue is NMT 2 mg (0.2%).

Riboflavin $C_{17}H_{20}N_4O_6$ [See monograph, Part I]

Riboflavin sodium phosphate $C_{17}H_{20}N_4NaO_4P$ [See monograph, Part I on Riboflavin Sodium Phosphate]

Rose bengal $C_{20}H_2Cl_4I_4Na_2O_5$ [Special class] Reddish brown powder, showing a reddish purple color when dissolved in water.

Rose bengal TS Dissolve 1 g of rose bengal in water to make 100 mL.

Saccharated pepsin [See monograph, Part I]

Saffron powder [See monograph, Part II]

Salicylaldazine $C_{14}H_{12}N_2O_2$ Dissolve 0.30 g of hydrazinium sulfate in 5 mL of water. To this solution, add 1 mL of acetic acid (100) and 2 mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well to mix, and allow to stand until a yellow precipitate is produced. Extract twice with 15 mL of dichloromethane, combine all the dichloromethane extracts, and add 5 g of anhydrous sodium sulfate. Shake to mix, decant or filter, and evaporate the dichloromethane in the supernatant or filtrate. Dissolve the residue in a warmed mixture of toluene and methanol (6 : 4), and cool. Take the crystals produced

by filtering, and dry in a desiccator (in vacuum, silica gel) for 24 hours. Yellow crystalline powder.

Melting point: 213 ℃ to 219 ℃

Purify: Related substance: Dissolve 90 mg of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100mL, and perform the test with this solution as directed in the Purity (6) under Povidone; any spot other than the principal spot does not appear.

Salicylaldehyde HOC_6H_4CHO [Special class].

Salicylic acid HOC_6H_4COOH [Special class].

Salicylic acid TS Dissolve 0.1 g of salicylic acid in 10 mL of sulfuric acid. Prepare before use.

Saturated sodium sulfate solution Weigh about 50 g of sodium sulfate, add 400 mL of water to a 500-mL volumetric flask, and stir vigorously. Add sodium sulfate in small amounts until the solution is saturated and crystals precipitate (dextran 70, hypromellose 2910·dextran 70 eye drops).

Scopolamine hydrobromide $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ [See monograph, Part I]

Sea sand [Special class]

sec-Butanol See 2-butanol.

Selenous acid H_2SeO_3 Colorless to white crystals that are hygroscopic.

Identification (1) Dissolve 0.2 g of selenous acid in 20 mL of water, and use this as the test solution. Add 2 mL of tin(II) chloride solution to 10 mL of this solution; the resulting solution appears brown.

(2) To 10 mL of the test solution obtained in (1), add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS; a brown color is produced.

Packaging and storage: Preserve in light resistant, tight containers.

Selenous acid-sulfuric acid TS Dissolve 50 mg of selenous acid in 10 mL of sulfuric acid.

Semicarbazide acetate TS Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30 mL of methanol in a flask, heat on a water bath for 2 hours, cool to 20°C, and filter. To the filtrate, add methanol to make 100 mL. Preserve this solution in a cold place. Do not use the solution showing a yellow color.

L-Serine $C_3H_7NO_3$ [Special class]

Sesame oil [See monograph, Part II].

Silica for chromatography Prepared for chromatography.

Silica gel An amorphous glassy granule with partial silicic acid hydate. For drying agents, some show changes in color due to moisture adsorption. If it is dried at 110 °C, it returns to its original color.

Loss on ignition: NMT 6% (2 g, 950 ± 50 °C).

Content: NLT 31%. Accurately weigh about 10 g of silica gel in a weighing bottle, open the lid, put it in a container with a humidity of 80% with sulfuric acid having a specific gravity of 1.19 for 24 hours. Weigh the mass and measure the weight increase of the sample.

Silica gel for gas chromatography A silica gel in good quality, prepared for gas chromatography.

Silica gel for liquid chromatography A silica gel prepared in good quality for liquid chromatography.

Silica gel for thin layer chromatography A high quality silica gel prepared for thin layer chromatography.

Silica gel for thin layer chromatography (with fluorescent indicator) A dimethylsilyl silica gel for thin-layer chromatography containing fluorescent indicator.

Silica gel for thin layer chromatography (with mixed fluorescent indicator) A silica gel for thin-layer chromatography containing fluorescent indicator.

SIlicon dioxide-tungstic acid hydrate $SiO_2 \cdot 12WO_3 \cdot 26H_2O$

Silicone oil A clear, colorless liquid with no odor.

Viscosity: 50 to 100 mm^2/s

Silicone resin A pale gray translucent viscous liquid or porridge-like substance and almost odorless.

Refractive index and viscosity: Place about 15 g of silicone resin in a Soxhlet extractor, extract it with 150 mL of carbon tetrachloride for 3 hours, and evaporate the extract on a water bath. The viscosity of the resulting solution is 100 to 1100 mm^2/s (25 ℃), and the refractive index is 1.400 to 1.410 (25 ℃).

Specific gravity: 0.98 to 1.02

Loss on drying of extraction residue (refractive index and viscosity): 0.45 to 2.25 g (100 ℃, 1 hour)

Silver chromate-saturated potassium chromate TS Dissolve 5 g of potassium chromate with 50 mL of water, add silver nitrate TS until a pale red precipitate is formed, filter it, and to the filtrate, add water to make 100 mL.

Silver diethyldithiocarbamate See silver *N,N*-diethyldithiocarbamate.

Silver *N,N*-diethyldithiocarbamate $C_5H_{10}AgNS_2$ [Special class]

Silver *N,N*-diethyldithiocarbamate $C_5H_{10}AgNS_2$ [Special class]

Silver nitrate $AgNO_3$ [Special Class]

Silver nitrate TS Dissolve 17.5 g of silver nitrate in water to make 1000 mL (0.1 mol/L). Store away from light.

Silver nitrate-ammonia TS Dissolve 1 g of silver nitrate in 20 mL of water, and add ammonia TS dropwise with stirring for mixing until the precipitate is almost entirely dissolved. Preserve in a stoppered, light-resistant container.

Skim-milk powder solution, 13% Dissolve 13 g of skim milk, accurately weighed, in 50 mL of water at 50 ℃ to 60 ℃, add 10 mL of 0.2 mol/L calcium acetate solution, shake vigorously for mixing, adjust the pH to 5.8 with 0.1 mol/L citric acid TS, and

add water to make 100 mL (gastropylore powder).

Soda lime [for carbon dioxide absorption]

Sodium Na [Special class]

Sodium 1-decanesulfonate $C_{10}H_{21}NaO_3S$ White powder.

Purity *Clarity and color of solution*: Dissolve 1.0 g of sodium 1-decanesulfonate in 20 mL of water; the resulting solution is clear and colorless.

Loss on drying: NMT 3.0% (1 g, 105 ℃, 3 hours)

Content: NLT 98.0%.

Assay: Weigh accurately about 0.45 g of sodium 1-decanesulfonate, dissolve in 50 mL of water, and pass through a column about 1.2 cm in internal diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion exchange resin (0.3 to 1.0 mm, H type) for column chromatography at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make necessary corrections.

Each mL of sodium hydroxide VS
= 24.43 mg of $C_{10}H_{21}NaO_3S$

Sodium 3-(trimethylsilyl)-propionic acid-d_4 for nuclear magnetic resonance spectroscopy $(CH_3)_3SiCD_2CD_2COONa$ Prepared for nuclear magnetic resonance spectroscopy.

Sodium acetate See sodium acetate trihydrate.

Sodium acetate buffer solution Weigh 86.5 g sodium hydroxide and 10.3 g of sodium acetate trihydrate, add water, and dissolve to make 1000 mL.

Sodium acetate trihydrate $CH_3COONa \cdot 3H_2O$ [Special class]

Sodium acetate TS Dissolve 13.6 g of sodium acetate trihydrate in water to make 100 mL (1 mol/L).

Sodium acetate-acetone TS Dissolve 8.15 g of sodium acetate trihydrate and 42 g of sodium chloride in 100 mL of water, and add 68 mL of 0.1 mol/L hydrochloric acid, 150 mL of acetone and water to make 500 mL.

Sodium acetate-sodium hydroxide TS Dissolve 10.3 g of sodium acetate trihydrate and 86.5 g of sodium hydroxide in water to make 1 L.

Sodium acetate, anhydrous CH_3COONa [sodium acetate (anhydrous), Special class]

Sodium ammonium hydrogen phosphate See sodium ammonium hydrogen phosphate tetrahydrate.

Sodium ammonium hydrogen phosphate tetrahydrate $NaNH_4HPO_4 \cdot 4H_2O$ [Special Class]

Sodium azide NaN_3 Colorless, hexaform crystals. It is freely soluble in water and decomposes to sodium and nitrogen when heated.

Density: 1.846

Sodium barbital $C_8H_{11}N_2NaO_3$ White crystals or crystalline powder. It is odorless and have a bitter taste. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in ether.

pH: The pH of a solution of sodium barbital (1 in 200) is between 9.9 and 10.3.

Loss on drying: NMT 1.0% (1 g, 105℃, 4 hours).

Content: NLT 98.5%.

Assay: Weigh accurately about 0.5 g of sodium barbital, previously dried, transfer to a separatory funnel, dissolve with 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract three times with 25 mL of chloroform, combine all the chloroform extracts, wash twice with 5 mL of water, and extract the washings twice with 10 mL each of chloroform. Combine the previous and these chloroform extracts, and filter into a Erlenmeyer flask. Wash the filtrate three times with 5 mL each of chloroform, combine the filtrate and the washings, add 10 mL of ethanol (95), and titrate with 0.1 mol/L potassium hydroxide-ethanol VS (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). The endpoint of the titration is when the yellow color of this solution turns to pale blue then finally to purple. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 20.618 mg of $C_8H_{11}N_2NaO_3$

Sodium benzoate $C_7H_5NaO_2$ [See monograph, Part I]

Sodium bicarbonate $NaHCO_3$ [Sodium bicarbonate (Sodium bicarbonate), Special Class]

Sodium bicarbonate for pH measurement See sodium bicarbonate, for pH measurement.

Sodium bicarbonate TS To 5.0 g of sodium bicarbonate, add water to dissolve and make 100 mL.

Sodium bicarbonate, for pH measurement $NaHCO_3$ [Sodium bicarbonate (Sodium bicarbonate), for pH measurement]

Sodium biphenyl See sodium biphenyl.

Sodium biphenyl $C_{12}H_9Na$ Use as a solution prepared by dissolving sodium biphenyl in a mixture of dimethoxyethane and toluene or xylene to make 10 w/w% ~ 30 w/w%. This solution is a dark green, viscous liquid. Preserve with care as this solution deteriorates at a rate of about 10% per month. Prepare freshly before use.

Activity: Place 20 mL of dried toluene in a flask equipped with a magnetic stirring bar and a weight buret having a hole for outlet insertion and a stopper. Add a certain amount of sodium biphenyl until the mixture exhibits a blue color, and titrate with n-amyl alcohol, contained in a weight buret, to the disappearance of the blue color. (Disregard the amounts of sodium biphenyl and n-amyl alcohol used in this adjustment.) Weigh accurately the mass of the weight buret containing the n-amyl alcohol. Transfer the contents, equivalent to 1 vial of well-mixed sample to the flask, and titrate quickly with n-amyl alcohol to the disappearance of the blue color. Weigh the mass of the buret to determine the weight of n-amyl alcohol consumed, and calculate the activity accoridng to the following equation,

Activity (milli equivalent per 1 vial) = 11.25 M

M : The mass of n-amyl alcohol consumed

Activity is NLT 10%.

Iodine content: Add 10 mL of sodium biphenyl to a 125-mL separatory funnel, containing 5 mL of toluene, fitted with a plastic stopcock, and shake vigorously for 2 minutes. Extract three times with 10 mL of dilute phosphoric acid (1 in 3), collect the lower layers in a 125-mL iodine flask. To this solution, add sodium hypochlorite TS dropwise until the solution turns brown, and then add another 0.5mL. Shake intermittently for 3 minutes, add 5 mL of freshly prepared, saturated phenol solution. Add 1 g of potassium iodide, shake to mix for 30 seconds, add 3 mL of starch TS, and titrate with 0.1 mol/L sodium thiosulfate VS; the consumed amount NMT 0.1 mL.

Sodium bismuth trioxide $NaBiO_3$ Yellowish brown powder.

Identification (1) To 10 mg of sodium bismuth trioxide, add 5 mL of a solution of manganese(II) nitrate (4 in 125) and 1 mL of dilute nitric acid (1 in 3), and shake vigorously for 10 seconds; the solution exhibits a purple color.

(2) Dissolve 10 mg of sodium bismuth trioxide in 2 mL of dilute hydrochloric acid (1 in 2); the solution responds to the Qualitative Analysis (1) for sodium salt.

Sodium bismuthate See sodium bismuth oxide.

Sodium bisulfite $NaHSO_3$ [Special class]

Sodium bisulfite $Na_2S_2O_5$ [First Class]

Sodium bisulfite TS Dissolve 10 g of sodium bisulfite in water to make 30 mL. Prepare before use.

Sodium bisulfite TS Dissolve 0.1 g of sodium bisulfite in 10 mL of 1 mol/L hydrochloric acid TS and add acetone to make 100 mL.

Sodium borate See sodium tetraborate decahydrate.

Sodium borate for pH measurement See sodium tetraborate decahydrate, for pH measurement.

Sodium borate solution, 0.1 mol/L, pH 9.3 Dissolve 38.1 g of sodium borate with water to make 1 L (sodium alendronate hydrate).

Sodium borate, for pH measurement See sodium tetraborate decahydrate, for pH measurement.

Sodium borohydride $NaBH_4$ White to grayish white crystal, powder, or masses. Sodium borohydride is freely soluble in water.

Content: NLT 95%.

Assay: Weigh accurately 0.25 g of Sodium borohydride, dissolve in 20 mL of diluted sodium hydroxide TS (3 in 10), and add water to make exactly 500 mL. Pipet 20 mL of this solution, transfer it into a glass-stoppered iodine flask, and cool on ice. Add exactly 40 mL of iodine TS, allow the mixture to stand at a dark place for 10 minutes. Add exactly 10 mL of diluted sulfuric acid (1 in 6), and perform back titration of the resulting solution with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS

= 0.4729 mg of $NaBH_4$

Sodium bromide NaBr [Special class].

Sodium carbonate See sodium carbonate decahydrate.

Sodium carbonate (standard reagent) Na_2CO_3 [Standard reagent for volumetric analysis]

Sodium carbonate decahydrate $Na_2CO_3 \cdot 10H_2O$ [Special Class]

Sodium carbonate ethanol TS Add 75% ethanol to 1 mL of 20% sodium carbonate TS to make 100 mL (histidine hydrochloride).

Sodium carbonate for pH measurement See sodium carbonate, for pH measurement.

Sodium carbonate solution See sodium carbonate solution, 0.4 mol/L.

Sodium carbonate solution, 0.4 mol/L Add water to 42.5 g of sodium carbonate to make 1 L (diastase and protease 500).

Sodium carbonate TS Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).

Sodium carbonate TS, 0.55 mol/L Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.

Sodium carbonate, anhydrous Na_2CO_3 [Sodium carbonate (anhydrous), Special Class]

Sodium carbonate, for pH measurement Na_2CO_3 [Sodium carbonate (anhydrous), for pH measurement]

Sodium carboxymethyl cellulose solution Add 50 mL of water to 0.625 g of carboxymethylcellulose sodium (pharmacopoeia) (degree of polymerization about 500),previously dried at 105 ℃ for 4 hours and accurately weighed, dissolve by warming, cool, add 10 mL of pH 4.5 acetic acid-sodium acetate buffer solution and water to make 100 mL.

Sodium chloride NaCl [Special Class]

Sodium chloride (standard reagent) NaCl [Reference material for volumetric analysis].

Sodium chloride solution Dissolve 0.824 g of sodium chloride, accurately weighed, in water to make 1000 mL.

Sodium chloride solution, 0.2 mol/L Add water to 0.234 g of sodium chloride to make 20 mL (pancreatinI).

Sodium chloride TS Dissolve 10 g of sodium chloride in water to make 100 mL.

Sodium chloride TS, 0.1 mol/L Dissolve 6 g of sodium chloride in water to make 1000 mL.

Sodium citrate See trisodium citrate dihydrate.

0.05 mol/L sodium citrate-0.05 mol/L sodium phosphate

buffer solution (pH 8.0) mixture Weigh 14.7 g of sodium citrate and 7.05 g of sodium monohydrogen phosphate, dissolve in 900 mL of water, and add phosphoric acid to adjust the pH to 8.0. Add water to make 1000 mL (sodium alendronate hydrate).

Sodium citrate, 0.1 mol/L Dissolve 29.4 g of sodium citrate in water to make 1000 mL (sodium alendronate hydrate).

Sodium cobaltnitrite TS Dissolve 10 g of sodium cobaltnitrite in water to make 50 mL. If necessary, filter it. Prepare before use.

Sodium cyanide NaCN [Special class].

Sodium cyanide TS Dissolve 9 g of sodium cyanide in about 80 mL of water, adjust the pH to 5.5 with careful stirring with acetic acid (100), and add water to make 100 mL. Prepare before use in a fume hood with caution.

Sodium deoxycholate $C_{24}H_{39}NaO_4$ White crystalline powder, having no odor.

Identification: Determine the infrared spectrum of sodium deoxycholate as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 3400 cm^{-1}, 2940 cm^{-1}, 1562 cm^{-1}, and 1408 cm-1.

Purity *Related substances*: Dissolve 0.1 g of sodium deoxycholate in 10 mL of methanol, and use this solution as the test solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use it as the standard solution. Perform the test with the test solution and the standard solution as directed under the Thin Layer Chromatography. Spot 10 μL each of the test solution and the standard solution on the thin plate made of silica gel for thin-layer chromatography. Then, develop the plate with a mixture of 1-butanol, methanol, and acetic acid (100) (80 : 40 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105 ℃ for 10 minutes; the spots other than the principal spot obtained from the test solution are not more intense than the spots from the standard solution.

Sodium diethyldithiocarbamate See sodium *N,N*-diethyldithiocarbamate trihydrate.

Sodium *N,N*-diethyldithiocarbamate trihydrate $(C_2H_5)_2NNaCS_2 \cdot 3H_2O$ [Special class]

Sodium dihydrogen phosphate See sodium dihydrogen phosphate dihydrate.

Sodium dihydrogen phosphate buffer solution, pH 3.0 Dissolve 6.2 g of sodium dihydrogen phosphate dihydrate in water to make 2000 mL and add phosphoric acid (1 in 10) to adjust the pH to 3.0 (magnesium dimecrotate).

Sodium dihydrogen phosphate dihydrate $NaH_2PO_4 \cdot 2H_2O$ [Special Class]

Sodium dihydrogen phosphate monohydrate NaH2PO4·H2O [Special Class]

Sodium dihydrogen phosphate TS, 0.05 mol/L Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium dihydrogen phosphate TS, 0.05 mol/L, pH 2.6 Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to exactly 2.6 with phosphoric acid, and add water to make 1000 mL.

Sodium dihydrogen phosphate TS, 0.05 mol/L, pH 3.0 To a 500 mL solution made by dissolving 3.45 g of sodium dihydrogen phosphate dihydrate in water, add a 500 mL solution prepared by dissolving 2.45 g of phosphoric acid in water until the pH is adjusted to 3.0.

Sodium dihydrogen phosphate TS, 0.1 mol/L Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, adjust the pH to exactly 5.8 with sodium hydroxide TS, and add water to make 500 mL.

Sodium dihydrogen phosphate TS, 0.1 mol/L, pH 3.0 Dissolve 15.60 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

Sodium dihydrogen phosphate TS, 2 mol/L Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium dodecyl sulfate $C_{12}H_{25}SO_4Na$ Bright yellow powder.

Sodium dodecylbenzenesulfonate $C_{18}H_{29}SO_3Na$ White crystalline powder or mass.

pH: The pH of a solution of 0.5 g of sodium dodecylbenzenesulfonate in 50 mL of freshly boiled and cooled water is 5.0 to 7.0. However, determine the pH at 25 ℃ by passing through nitrogen while stirring.

Loss on drying: NMT 0.5% (1 g, 105 ℃, 2 hours).

Content: NLT 99.0%.

Assay: Weigh accurately about 40 mg of sodium dodecylbenzenesulfonate, previously dried, and perform the test as directed in the Assay of Sulfur under the Oxygen Flask Combustion, using a mixture of 20 mL of water and 2 mL of hydrogen peroxide (30) water as an absorbent.

Each mL of 0.005 mol/L barium perchlorate VS
= 1.743 mg of $C_{18}H_{29}SO_3Na$

Sodium fluoride NaF [Special class]

Sodium fluoride (standard reagent) NaF [standard substance for volumetric analysis].

Sodium fluoride TS See sodium fluoride TS.

Sodium fluoride TS Dissolve 0.5 g of sodium fluoride in 100 mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.

Sodium formate-sodium hydroxide TS Prepare a mixture of equal amounts of sodium formate solution (2 in 10) and sodium hydroxide solution (2 in 10), and evaporate to dryness on a water bath.

Sodium heptanesulfonate $C_7H_{15}NaO_3S$ White crystals or crystalline powder.

Purity: *Clarity and color of solution*: Dissolve 1.0 g of Sodium Heptanesulfonate in 10 mL of water; the resulting solution is clear and colorless.

Loss on drying: NMT 3.0% (1 g, 105°C, 3 hours).

Content: NLT 98.0%

Assay: Weigh accurately about 0.4 g of sodium heptanesulfonate, previously dried, dissolve in 50 mL of water, transfer into a chromatographic column, prepared by packing a chromatographic tube, 9 mm in internal diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600 μm in particle diameter, H type), and wash at a flow rate of about 4 mL per minute. Next, wash the chromatographic column with 150 mL of water at a rate of about 4 mL per minute. Combine the washings with the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) However, the endpoint of titration is when the yellow color of the solution changes to blue.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.225 mg of $C_7H_{15}NaO_3S$

Sodium 1-heptanesulfonate $C_7H_{15}NaO_3S$ White crystals or crystalline powder.

Purity *Clarity and color of solution*: Dissolve 1.0 g of sodium 1-heptanesulfonate in 10 mL of water; the resulting solution is clear and colorless.

Loss on drying: NMT 3.0% (1 g, 105°C, 3 hours).

Content: NLT 98.0%

Assay: Weigh accurately about 0.4 g of sodium 1-heptanesulfonate, previously dried, dissolve in 50 mL of water, transfer into a chromatographic column, prepared by packing a chromatographic tube, 9 mm in internal diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600 μm in particle diameter, H type), and wash at a flow rate of about 4 mL per minute. Next, wash the chromatographic column with 150 mL of water at a rate of about 4 mL per minute. Combine the washings with the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) However, the endpoint of titration is when the yellow color of the solution changes to blue.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.225 mg of $C_7H_{15}NaO_3S$

Sodium 1-heptanesulfonate monohydrate $C_7H_{15}NaO_3S \cdot H_2O$ [First class]

Sodium 1-hexanesulfonate $C_6H_{13}NaO_3S$ [Special class]

Sodium hexanesulfonate buffer solution, 0.005 mol/L, pH 6.0 Dissolve 0.94 g of sodium 1-hexanesulfonate in water and adjust the pH to 6.0, then add water to make 1000 mL.

Sodium hexanitrocobaltate(III) $Na_3Co(NO_2)_6$ [Special class]

Sodium hexanitrocobaltate(III) TS Dissolve 10 g of sodium hexanitrocobaltate(III) in water to make 50 mL, and Filter, if necessary. Prepare before use.

Sodium hydrogen tartrate See sodium hydrogen tartrate monohydrate

Sodium hydrogen tartrate monohydrate $NaHC_4H_4O_6 \cdot H_2O$ [Special Class]

Sodium hydrogen tartrate TS Dissolve 1 g of sodium hydrogen tartrate monohydrate in water to make 10 mL (0.5 mol/L). Prepare before use.

Sodium hydrosulfide $Na_2S_2O_4$ A colorless liquid, having a characteristic odor.

Sodium hydroxide NaOH [Special class].

Sodium hydroxide TS Dissolve 4.3 g of sodium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

Sodium hydroxide TS 0.1 mol/L for bacterial endotoxins test See sodium hydroxide TS, 0.1 mol/L, for bacterial endotoxins test.

Sodium hydroxide TS, 0.01 mol/L Dilute 10 mL of sodium hydroxide TS with water to make 1000 mL. Prepare before use.

Sodium hydroxide TS, 0.02 mol/L Dilute 10 mL of 0.2 mol/L sodium hydroxide TS with water to make 100 mL. Prepare before use.

Sodium hydroxide TS, 0.05 mol/L To 10 mL of 0.5 mol/L sodium hydroxide TS, add water to make 100 mL. Prepare before use.

Sodium hydroxide TS, 0.1 mol/L Dilute 10 mL of sodium hydroxide TS with water to make 100 mL. Prepare before use.

Sodium hydroxide TS, 0.1 mol/L, for bacterial endotoxins test Dissolve 4.3 g of sodium hydroxide in water for bacterial endotoxins test to make 1000 mL.

Sodium hydroxide TS, 0.2 mol/L Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use.

Sodium hydroxide TS, 0.5 mol/L Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 1 mol/L Dissolve 4.3 g of sodium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 10 mol/L Dissolve 440 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 2 mol/L Dissolve 8.6 g of sodium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 4 mol/L Dissolve 168 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 5 mol/L Dissolve 220 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 8 mol/L Dissolve 336 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, dilute Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use (0.1 mol/L).

Sodium hydroxide-dioxane TS Dissolve 0.80 g of sodium hydroxide in a mixture of 1,4-dioxane and water (3 : 1) to make 100 mL.

Sodium hydroxide-ethanol TS, 0.1 mol/L Add ethanol to 3.3 g of 10 mol/L sodium hydroxide solution to make 250 mL.

Sodium hydroxide-glycine buffer solution, pH 12.0 Dissolve 585 mg of sodium chloride and 750.0 mg of glycine in water to make 1 L. Mix 180.4 mL of this solution with 21.6 mL of 0.1 mol/L sodium hydroxide TS.

Sodium hydroxide-methanol TS Dissolve 4 g of sodium hydroxide in methanol to make 100 mL. Centrifuge this solution, collect 50 mL of the supernatant, and add methanol to make 500 mL. Prepare before use.

Sodium hydroxide-methanol TS, 0.5 mol/L Dissolve 20 g of sodium hydroxide in methanol to make 100 mL. Centrifuge this solution, collect 50 mL of the supernatant, and add methanol to make 500 mL. Prepare before use.

Sodium hydroxide-potassium cyanide buffer solution, pH 12.8 Dissolve 80 g of sodium hydroxide and 5.2 g of potassium cyanide in water to make 1000 mL.

Sodium hypobromate NaBrO [First Class]

Sodium hypobromite TS Add 25 mL of water and 25 mL of sodium carbonate TS to 8 mL of bromide TS. Prepare before use.

Sodium hypochlorite TS Prepare the solution by passing chlorine into sodium hydroxide in aqueous solution while cooling with ice, so that the solution contains 5% of sodium hypochlorite. Prepare before use.

Sodium hypochlorite TS for ammonium test See sodium hypochlorite TS, for ammonium test.

Sodium hypochlorite TS, for ammonium test A clear, colorless to pale greenish yellow solution prepared by passing chlorine into an aqueous solution of sodium hydroxide or sodium carbonate, having the odor of chlorine.

Content: NLT 4.2 w/v% as hypochlorous acid.

Assay: Pipet 10 mL of Sodium hypochlorite, for ammonium test and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution to a glass-stoppered flask, add 90 mL of water, then add 2 g of potassium iodide and 6 mL of diluted acetic acid (1 in 2), stopper tightly, shake well, and allow to stand in a dark place. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate TS (indicator: 3 mL of starch TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.7221 mg NaClO.

Sodium hypochlorite-sodium hydroxide TS To a volume of sodium hypochlorite TS for ammonium test equivalent to 1.05 g of sodium hypochlorite, add 15 g of sodium hydroxide and water to make 1000 mL. Prepare before use.

Sodium iodate $NaIO_3$ [Special Class]

Sodium iodide NaI [Special Class]

Sodium lactate $C_6H_5NHNH_2$ [Special class]

Sodium lactate solution, 0.1 mol/L See sodium lactate solution, 0.1 mol/L.

Sodium lauryl sulfate [See monograph, Part II]

Sodium metabisulfite See sodium pyrosulfite.

Sodium metabisulfite TS See sodium pyrosulfite TS.

Sodium metal See sodium.

Sodium methoxide CH_3ONa

Sodium molybdate See sodium molybdate dihydrate.

Sodium molybdate dihydrate $Na_2MoO_4 \cdot 2H_2O$ [Special class].

Sodium monohydrogen phosphate See disodium hydrogen phosphate dodecahydrate.

Sodium monohydrogen phosphate TS See disodium hydrogen phosphate TS.

Sodium monohydrogen phosphate TS, 0.05 mol/L See disodium hydrogen phosphate TS, 0.05 mol/L

Sodium monohydrogen phosphate TS, 0.2 mol/L See disodium hydrogen phosphate TS, 0.2 mol/L

Sodium monohydrogen phosphate TS, 0.5 mol/L See disodium hydrogen phosphate TS, 0.5 mol/L

Sodium monohydrogen phosphate-citric acid buffer solution, 0.05 mol/L, pH 6.0 See sodium monohydrogen phosphate-citric acid buffer solution, 0.05 mol/L, pH 6.0.

Sodium monohydrogen phosphate-citric acid buffer solution, pH 4.0 Mix 122 mL of 0.1 mol/L citric acid solution and 77 mL of 0.2 mol/L sodium monohydrogen phosphate TS to adjust the pH to 4.0 (pridinol mesilate).

Sodium monohydrogen phosphate-citric acid buffer solution, pH 4.5 See sodium monohydrogen phosphate-citric acid buffer solution, pH 4.5.

Sodium monohydrogen phosphate-citric acid buffer solution, pH 5.4 See sodium monohydrogen phosphate-citric acid buffer solution, pH 5.4.

Sodium monohydrogen phosphate-citric acid buffer solution, pH 6.0 See sodium monohydrogen phosphate-citric acid buffer solution, pH 6.0.

Sodium monohydrogen phosphate, anhydrous See disodium hydrogen phosphate, anhydrous.

Sodium monohydrogen phosphate, anhydrous, for pH measurement See disodium hydrogen phosphate, anhydrous, for pH

measurement.

Sodium 3-(*N*-morpholino)propanesulfonate $C_7H_{14}NNaO_4S$ [Special class]

Sodium 2-naphthalenesulfonate $C_{10}H_7NaO_3S$ Pale brown crystal or powder.

Content: NLT 98.0%.

Sodium naphthoquinone sulfonate TS Dissolve 0.25 g of sodium β-naphthoquinonesulfonate in methanol to make 100 mL.

Sodium *β*-Naphthoquinonesulfonate $C_{10}H_5NaO_5S$ Yellow to yellowish orange crystal or crystalline powder. Soluble in water and practically insoluble in ethanol (95).

Loss on drying: NMT 2.0% (1 g, in vacuum, 50 °C).

Residue on ignition: 20.0 to 30.0% (1 g, after drying).

Sodium nitrate $NaNO_3$ [Special Class]

Sodium nitrite $NaNO_2$ [Special class]

Sodium nitrite solution Dissolve 5 g of sodium nitrite in water to make 100 mL (doxazosin mesilate).

Sodium nitrite TS Dissolve 10 g of sodium nitrite in water to make 100 mL. Prepare before use.

Sodium nitroprusside See sodium pentacyanonitrosylferrate(III) dihydrate.

Sodium nitroprusside See sodium pentacyanonitrosylferrate(III) dihydrate.

Sodium nitroprusside TS See sodium pentacyanonitrosylferrate(III) TS.

Sodium nitroprusside TS See sodium pentacyanonitrosylferrate(III) TS.

Sodium 1-octanesulfonate $CH_3(CH_2)_7SO_3Na$ White crystals or powder.

Residue on ignition: 32.2% to 33.0% (1.0 g).

Sodium octanesulfonate solution, 0.005 mol/L Dissolve 1.08 g of sodium 1-octanesulfonate in water to make 1000 mL (nicametate citrate tablet).

Sodium oxalate (standard reagent) $Na_2C_2O_4$ [Standard reagent for volumetric analysis]

Sodium *p*-toluenesulfonchloramide trihydrate $C_7H_7ClNNaO_2S\cdot 3H_2O$

Sodium pentacyanoamine ferroate $Na_3[Fe(CN)_5NH_3]\cdot xH_2O$ [First class]

Sodium pentacyanonitrosylferrate(III) dihydrate $Na_2[Fe(CN)_5(NO)]\cdot 2H_2O$ [Special class]

Sodium pentacyanonitrosylferrate(III) TS Dissolve 1 g of sodium pentacyanonitrosylferrate(III) dihydrate in water to make 20 mL. Prepare before use.

Sodium 1-pentane sulfonate $C_5H_{11}NaO_3S$ White crystals or crystalline powder. Freely soluble in water, and practically insoluble in acetone.

Clarity and color of solution: Dissolve 1.0 g of Sodium 1-Pentane Sulfonate in 10 mL of water: the solution is colorless and clear.

Water: NMT 3.0% (0.2 g).

Content: NLT 99.0%, calculated on the anhydrous basis.

Assay: Weigh accurately about 0.3 g of Sodium 1-Pentane Sulfonate, dissolve in 50 mL of water, put in a chromatographic column [made by injecting 10 mL of 425 to 600 μL of strongly acidic ion exchange resin (H type) into a chromatographic tube with an internal diameter of 9 nm and a height of 160 mm], and elute at a rate of about 4 mL per minute. Next, wash the chromatographic column with 50 mL of water at a rate of 4 mL per minute.. Combine the washings with the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) The endpoint of titration is when the yellow color of the liquid changes to blue.

Each mL of 0.1 mol/L sodium hydroxide VS
= 17.420 mg of $C_5H_{11}NaO_3S$

Sodium perchlorate $NaClO_4\cdot H_2O$ [Special class]

Sodium periodate $NaIO_4$ [Special class]

Sodium periodate TS Dissolve 60.0 g of sodium periodate in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. If this solution is not clear, filter through a glass filter. Preserve in light-resistant containers.

Sodium peroxide Na_2O_3 [Special class]

Sodium phenol-nitroprusside sodium TS See sodium phenol-pentacyanonitrosylferrate(III) TS

Sodium phenol-pentacyanonitrosylferrate(III) TS Dissolve 5 g of phenol and 25 mg of sodium pentacyanonitrosylferrate(III) dihydrate in water to make 500 mL. Preserve in a cold, dark place.

Sodium *p*-Phenolsulfonate $C_6H_5O_4NaS\cdot 2H_2O$ Sodium *p*-Phenolsulfonate is a white to pale yellow crystal or crystalline powder, and has a characteristic odor.

Identification (1) Add 1 drop of iron(III) chloride TS to 10 mL of the aqueous solution (1 in 100) of Sodium *p*-Phenolsulfonate; the resulting solution exhibits a violet color.

(2) Determine the absorption spectrum of aqueous solution of Sodium *p*-Phenolsulfonate (1 in 5000) as directed under the Ultraviolet-visible Spectroscopy; it exhibits maxima at 269 nm to 273 nm and at 276 nm to 280 nm of wavelengths.

Purity *Clarity and color of solution*: Dissolve 1.0 g of Sodium *p*-Phenolsulfonate in 25 mL of water; the resulting solution is clear and colorless.

Content: NLT 90.0%

Assay: Weigh accurately about 0.5 g of sodium *p*-phenolsulfonate, dissolve in 50 mL of water. Elute it into a chromatographic column[made by injecting 20 mL of strongly acidic ion exchange resin (H type) for 150 to 300 μm column chromatography into a chromatograph tube with an internal diameter of about 1 cm and a height of about 30 cm.] Next, wash the chromatographic column with water until the washings are no longer acidic. Combine the washings with the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromocresol

green-methyl red TS). Separately, weigh accurately 0.5 g of Sodium *p*-phenolsulfonate, dissolve in 50mL of water, and titrate with 0.1 mol/L sodium hydroxide VS.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.219 mg of $C_6H_5O_4NaS \cdot 2H_2O$

Sodium phosphate See trisodium phosphate dodecahydrate.

Sodium phosphate buffer solution, 0.1 mol/L, pH 6.3 Dissolve 11.999 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 1000 mL.

Sodium phosphate dibasic TS See disodium hydrogen phosphate TS.

Sodium phosphate solution, pH 8.0, 15 mmol/L Add 1 mL of 85% phosphoric acid to 1000 mL of water and add 50% sodium hydroxide solution to adjust the pH to 8.0.

Sodium phosphate TS Dissolve 5.68 g of anhydrous disodium hydrogen phosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium pyrosulfite $Na_2S_2O_5$ [See monograph]

Sodium pyrosulfite TS Dissolve 0.1 g of sodium pyrosulfite in 10 mL of 1 mol/L hydrochloric acid TS, and add acetone to make 100 mL.

Sodium salicylate HOC_6H_4COONa [Special class].

Sodium salicylate-sodium hydroxide TS Dissolve 1 g of sodium salicylate in 0.01 mol/L sodium hydroxide to make 100 mL.

Sodium selenite Na_2SeO_3 A white crystalline powder.

Identification. (1) Dissolve 1.0 g of Sodium Selenite in 100 mL of water, and use this as the test solution. To 10 mL of this solution, add 2 mL of tin(II) chloride TS: a red precipitate is produced.

(2) The test solution obtained in (1) responds to the Qualitative Analysis (1) for sodium salt.

Packaging and storage: Preserve in light resistant, tight containers.

Sodium *p*-styrenesulfonate $C_8H_7NaO_3S$ White crystal or crystalline powder. Freely soluble in water, slightly soluble in ethanol (99,5), and practically insoluble in ether. Recrystallize from diluted ethanol (1 in 2) and dry in vacuum.

Identification: Determine the infrared spectra of sodium *p*-styrenesulfonate as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption at the wavenumbers of about 1236 cm^{-1}, 1192 cm^{-1}, 1136 cm^{-1}, 1052 cm^{-1}, 844 cm^{-1}, and 688 cm^{-1}.

Purity: Perform the test with 10 µL of a solution of sodium *p*-styrenesulfonate (1 in 1000) as the test solution according to the operating conditions of the Liquid Chromatography under the Assay of Panipenem; any peaks other than panipenem are not observed.

Sodium sulfate See sodium sulfate decahydrate.

Sodium sulfate decahydrate $Na_2SO_4 \cdot 10H_2O$ [Special class]

Sodium sulfate, anhydrous Na_2SO_4 [Special class]

Sodium sulfide See sodium sulfide nonahydrate.

Sodium sulfide nonahydrate $Na_2S \cdot 9H_2O$ [Special class]

Sodium sulfide TS Dissolve 5 g of sodium sulfide nonahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or otherwise, dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate half the volume with cold hydrogen sulfide, and mix the remaining half volume. Preserve in well-filled, light-resistant bottles. Use within 3 months.

Sodium sulfide TS Dissolve sodium sulfide in a mixture of glycerin and water (35 : 65) to make the concentration of the solution 6% (diclofenac β-dimethylaminoethanol).

Sodium sulfide TS, for zinc test Dissolve 50 mg of sodium sulfide nonahydrate in water to make 100 mL (0.05%).

Sodium sulfite See sodium sulfite heptahydrate.

Sodium sulfite heptahydrate $Na_2SO_3 \cdot 7H_2O$ [Special class]

Sodium sulfite TS Dissolve 20 g of sodium bisulfite in water 100 mL. Prepare before use (lecithin).

Sodium sulfite, anhydrous Na_2SO_3 [sodium sulfate (anhydrous), Special class]

Sodium tartrate See sodium tartrate dihydrate.

Sodium tartrate dihydrate $C_4H_4Na_2O_6 \cdot 2H_2O$ [(+)Sodium tartrate dihydrate, Special Class]

Sodium taurocholate solution, 8% Dissolve 2 g of sodium taurocholate in 25 mL of water (use within 24 hours).

Sodium tetraborate decahydrate $Na_2B_4O_7 \cdot 10H_2O$ [Special class].

Sodium tetraborate decahydrate for pH measurement See Sodium tetraborate decahydrate, for pH measurement.

Sodium tetraborate decahydrate, for pH measurement [for pH measurement].

Sodium tetraborate-calcium chloride buffer solution, pH 8.0 Dissolve 0.572 g of sodium tetraborate decahydrate and 2.94 g of calcium chloride dihydrate in 800mL of freshly boiled and cooled water, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Sodium tetraphenylborate $(C_6H_5)_4BNa$ [Special Class]

Sodium thioglycolate $HSCH_2COONa$ [Special Class] Store in a cold, dark place, protected from light.

Sodium thiosulfate See sodium thiosulfate pentahydrate.

Sodium thiosulfate pentahydrate $Na_2S_2O_3 \cdot 5H_2O$ [Special Class].

Sodium thiosulfate solution, 0.05 mol/L Dissolve 12.409 g

of sodium thiosulfate in water to make 1000 mL (diastase and protease 500).

Sodium thiosulfate TS Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL (0.1 mol/L).

Sodium toluenesulfonchloramide trihydrate $C_7H_7ClNNaO_2S \cdot 3H_2O$ [*p*-sodium toluenesulfonchloramide trihydrate, Special Class]

Sodium toluenesulfonchloramide TS Dissolve 1 g of sodium toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare before use.

Sodium tridecanesulfonate $C_6H_6SO_3$ White crystals or powder.

Sodium tungstate(VI) dihydrate $Na_2WO_4 \cdot 2H_2O$ [Special Class]

Sodium, metal See sodium.

Soluble starch See starch, soluble.

Soluble starch solution, 0.5% Weigh accurately 0.5 g of soluble starch (dried), place in a small amount of water to mix, and slowly add the mixture in 500 mL of boiling water. Add 20 mL of acetate buffer solution, previously boiled for 5 minutes and then cooled, and water to make 100.0 mL (β-amylase).

Soluble starch solution, 1% Add 10 g of soluble starch, previously dried (120 ℃, 4 hours), to 50 mL of water, mix well while stirring, then slowly add 800 mL of boiling water while stirring, and wash the container well. Combine the washings with boiling water, and then boil while stirring well. Transfer the resulting solution to a 1000-mL volumetric flask, add water to make 1000 mL, and mix well (use within 24 hours after preparation) (pancreatin II).

Soluble starch solution, 1% Dissolve 1 g of soluble starch in 20 mL of water, add the solution in 30 mL of boiling water, gelatinize the solution for at least 5 minutes, and then add water to make 100 mL (diastase and protease 100, diastase and protease 500).

Soluble starch solution, 2% Dissolve 2 g of soluble starch in 20 mL of water, add the solution in 30 mL of boiling water, gelatinize for at least 5 minutes, cool, and then add 10 mL of pH 5.6 Macklebane buffer solution and water to make 100.0 mL (diastase and protease 100, diastase and protease 500).

Soluble starch TS Grind 1 g of soluble starch well with 10 mL of cold water for mixing, pour slowly into 90 mL of hot water while continuously stirring, boil gently for 3 minutes, and then cool. Prepare before use.

Soluble starch TS, 0.5% Weigh accurately an amount equivalent to 0.5 g of soluble starch, previously dried, and mix it with a small amount of water. Slowly transfer into 50 mL of boiling water, and boil for 5 minutes. After cooling, add 20 mL of acetate buffer solution, pH 4.8 and water to make exactly 100 mL (aspergillus).

Soluble starch TS, 0.5%, pH 5.0 Weigh accurately 1 g of soluble starch, dry at 105 ℃ for 4 hours, measure the weight loss, then weigh accurately an amount equivalent to 0.5 g of soluble starch, previously dried, and suspend it in a small amount of water. Then, slowly put it in 50 mL of boiling water. Boil for 5 minutes, cool, and then add 5 mL of 1 mol/L acetate buffer solution (pH 5.0) and water to make 100 mL. Prepare before use.

Soluble starch TS, 1%, pH 5.0 Weigh accurately an amount equivalent to 1.0 g of soluble starch, previously dried, mix it with 10 mL of water, and slowly put it in 50 mL of hot water while stirring. Boil for exactly 5 minutes, then cool, and add 25 mL of acetate buffer solution, pH 5.0 and water to make 100 mL. Prepare before use.

Solvent blue 19 Used for non-aqueous titration and changes from blue (basic) to violet (neutral) and then pink (acidic).

***D*-Sorbitol for gas chromatography** Prepared for gas chromatography.

***D*-Sorbitol** [See monograph, Part I]

Soybean oil [See monograph, Part II]

Soybean oil [See monograph, Part II]

Soybean peptone See soybean peptone.

Spherical porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075 μm, and the surface area is 500 m^2 to 600 m^2 per g.

Spherical styrene-divinylbenzene copolymer for liquid chromatography Prepared for liquid chromatography.

1.6% Stannic chloride-citric acid buffer solution, pH 5.0 Dissolve stannic chloride 1.6 g and citric acid hydrate 1.6 g in water 100 mL and adjust the pH to pH 5.0 with phosphoric acid or sodium hydroxide TS.

Stannous chloride See tin(II) chloride dihydrate.

Stannous chloride solution, acidic Dissolve 5 g of stannous chloride in 10 mL of hydrochloric acid, and add water to make 100 mL (ferrous chloride hydrate).

Stannous chloride TS Dissolve 5.6 g of stannous chloride in dilute hydrochloric acid (3 in 100) to make 50 mL, add a tin segment, and store in a brown bottle (soluble ferric pyrophosphate).

Stannous chloride TS See tin(II) chloride TS.

Stannous chloride TS, acidic See tin(II) chloride TS, acidic.

Stannous chloride-hydrochloric acid TS See tin(II) chloride-hydrochloric acid TS.

Stannous chloride-sulfuric acid TS See tin (II) chloride-sulfuric acid TS

Starch [Special Class]

Starch iodine TS Mix 1% starch TS, 1% iodine-4% potassium iodide solution, and acetic acid (100) in the ratio of 10 : 2 : 6.

Starch solution, Gelatinized Add 10 mL of water to 1 g of potato starch and heat on a water bath while shaking for mixing to make it a gelatinized solution (biodiastase 700G).

Starch TS Mix 1 g of starch with 10 mL of cold water, and pour the mixture slowly, with constant stirring, into 200 mL of boiling water. Boil the mixture until it becomes translucent, and allow to stand. Then, use the clear supernatant. Prepare before use.

Starch TS, 5% Mix 5 g of starch with water 100 mL by shaking well, and heat it on a water bath. Prepare before use.

Starch-sodium chloride TS Saturate starch TS with sodium chloride. Use within 5 to 6 days after preparation.

Starch, soluble [Special Class]

Stearyl alcohol [See monograph, Part II]

Strong ammonia water See ammonia water (28).

Strong hydrogen peroxide water See hydrogen peroxide (30).

Strongly acidic ion exchange resin for column chromatography Prepared for column chromatography.

Strongly acidic ion exchange resin for liquid chromatography See Strongly acidic ion exchange resin, for liquid chromatography.

Strongly acidic ion exchange resin for liquid chromatography (styrene-divinylbenzene copolymer sulfonic acid resin column type) Prepared for liquid chromatography.

Strongly acidic ion-exchange resin See ion-exchange resin, strong acid.

Strontium chloride $SrCl_2 \cdot 6H_2O$ [Special Class]

Styrene C_8H_8 Colorless, clear liquid.

Specific gravity *d:* 0.902 to 0.910

Purity: Perform the test with 1 μL of styrene as directed under the Gas Chromatography according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of styrene as directed under the percentage peak area method; NLT 99%.

Operating conditions

Detector: Thermal conductivity detector

Column: A column with an internal diameter of about 3 mm and a length of about 2 m, packed with diatomaceous earth for gas chromatography (180 to 250 μm), coated with polyethylene glycol 20 M for gas chromatography in a ratio of 10%.

Column temperature: Constant temperature around 100 °C.

Temperature of sample vaporization chamber: Constant temperature around 150 °C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of styrene is about 10 minutes.

Time span of measurement: About 2 times the retention time of styrene.

Substrate solution for peroxidase assay Dissolve 0.195 mL of hydrogen peroxide, 8.38 g of disodium hydrogen phosphate dodecahydrate, and 1.41 g of citric acid monohydrate in water to make 300 mL. Immediately prior to use, dissolve 15 mL of this solution with 13 mg *o*-phenylenediamine dihydrochloride.

Substrate solution, for fat digestion test Add 100 mL of Stoke emulsion, 80 mL of buffer solution, 20 mL of 8% sodium taurocholate solution and 95 mL of water in that order, and stir the mixture (prepare before use) (pancreas).

Substrate solution, for starch digestion test Dry the soluble starch at 120 ℃ and determine the water content. Mix 10 g of soluble starch (calculated on the dried basis) with about 50 mL of water in a beaker. Slowly transfer this solution into another beaker containing 800 mL of boiled water while stirring, and wash the beaker with about 50 mL of water. Heat while stirring to boiling, cool to 20 ℃, and then add water to make 1000 mL (prepare before use) (pancreas).

Substrate TS (1) for kallidinogenase assay Dissolve a suitable volume of H-D-valyl-L-leucy-L-arginine-4-nitroanilide dihydrochloride in 0.1 mol/L tris buffer solution, pH 8.0, to prepare a solution that contains 1 mg of H-D-valyl-L-leucy-L-arginine-4-nitroanilide dihydrochloride in 5 mL.

Substrate TS (2) for kallidinogenase assay Dissolve 17.7 mg of N-α-benzoyl-L-arginine ethyl hydrochloride with pH 8.0 0.1 mol/L tris buffer solution to make the total volume of 100 mL.

Substrate TS (3) for kallidinogenase assay Suspend 0.6 g of casein (milk) purified by Hammarsten method, in 80 mL of 0.05 mol/L disodium hydrogen phosphate TS and warm the suspension at 65 ℃ for 20 minutes to dissolve. After cooling, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 100mL. Prepare before use.

Succinic acid, anhydrous $C_4H_6O_4$ White or pale off-white crystals or flakes and is odorless. Soluble in water, freely soluble in hot water, and sparingly soluble in ethanol (95).

Melting point: 185 to 190 °C

Residue on ignition: NMT 0.10% (1 g).

Content: NLT 98.0%.

Assay: Weigh accurately about 1 g of Succinic acid, add 50 mL of water, and dissolve by warming. After cooling, titrate the resulting solution with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 59.05 mg of $C_4H_6O_4$

Sucrose $C_{12}H_{22}O_{11}$ [See monograph, Part II on Purified sucrose]

Sudan III $C_{22}H_{16}N_4O$ Reddish brown powder. Soluble in chloroform, acetic acid (100) and insoluble in water, ethanol, acetone, or ether.

Melting point: 170 to 190 ℃

Sudan III TS Dissolve 10 mg of Sudan III in 5 mL of ethanol, filter, and add 5 mL of glycerin to the filtrate. Prepare before use.

Sulbactam sodium for sulbactam penicillamine See sulbactam sodium, for sulbactam penicillamine.

Sulbactam sodium, for sulbactam penicillamine $C_8H_{10}NNaO_5S$ White to yellowish white crystalline powder. Freely soluble in water, and practically insoluble in ethanol (95).

Identification: Determine the infrared absorption spectrum of sulbactam sodium for sulbactam penicillamine as directed in the potassium bromide disk method under the Mid-infrared Spectroscopy; it exhibits absorption maxima at the wavenumbers of about 1780 cm-1, 1600 cm^{-1}, 1410 cm^{-1}, 1400 cm^{-1}, 1320 cm^{-1}, 1300 cm^{-1}, 1200 cm^{-1} and 1130 cm^{-1}.

Water: NMT 1.0% (0.5 g).

Content: Mot less than 875 μg/mg (calculated on the anhydrous basis)

Assay: Weigh accurately 0.10 g (potency) each of sulbactam sodium for sulbactam penicillamine and sulbactam RS, dissolve each in the mobile phase, add 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the test solution and the standard solution, respectively. Perform the test with 10 μL each of the test solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak area ratio Q_T and Q_S of each solution to the peak area of the internal standard solution.

$$\text{Amount (potency) of sulbactam } (C_8H_{10}NNaO_5S) = M_S \times Q_T/Q_S \times 1000$$

Ms: Amount of sulbactam RS

Internal standard solution—A solution of ethylparahydroxybenzoate in the mobile phase (7 in 1000).

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 3.9 mm in internal diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: Constant temperature around 35 ℃

Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammmonium hydroxide TS, add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

System suitability

System performance: Proceed with 10 μL of the standard solution according to the above operating conditions; sulbactam and the internal standard are eluted in this order with the the resolution being NLT 4.

System repeatability: Repeat the test 6 times with 10 μL each of the standard solution according to the above conditions; the relative standard deviation of the peak areas of sulbactam is NMT 1.0%.

Sulfamic acid (standard reagent) See amidosulfuric acid (standard reagent).

Sulfanilamide $H_2NC_6H_4SO_2NH_2$ [Special class]

Sulfanilamide for diazotization titration See sulfanilamide, for diazotization titration.

Sulfanilamide, for diaxotization titration $H_2NC_6H_4SO_2NH_2$ [For diazotization titration]

Sulfanilic acid $H_2NC_6H_4SO_2NH_2$ [Sulfanilamide, Special class]

Sulfanilic acid buffer solution Dissolve 2.5 g of sulfanilic acid and 4.0 g of anhydrous sodium acetate in 40 mL of water, and add ethanol to make the total amount 175 mL.

Sulfanilic acid TS, 1% Dissolve 1 g of sulfanilic acid in 10% hydrochloric acid to make 100 mL.

Sulfathiazole $C_9H_9N_3O_3S_2$ White crystalline powder
Melting point: 200 ℃ to 204 ℃

Sulfomolybdic acid TS Dissolve 2.5 g of ammonium molybdate in 20 mL of water by heating. Separately, dilute 28 mL of sulfuric acid with 50 mL of water. Mix the two solutions, and add water to make 100 mL. Preserve in a polyethylene bottle.

Sulfosalicylic acid dihydrate $C_7H_6O_6S \cdot 2H_2O$ [Special class]

Sulfosalicylic acid TS Dissolve 5 g of sulfosalicylic acid dihydrate in water to make 100 mL.

Sulfosalicylic acid TS, 10 w/v% Dissolve 1.0 g of sulfosalicylic acid dihydrate in water to make 10 mL.

Sulfur S [Special class]

Sulfur dioxide SO_2 Prepare by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

Sulfur dioxide detector column Melt-sealed glass column designed so that an appropriate adsorption filter and gas containing an iodine starch indicator, for detection of sulfur dioxide, can pass through (sulfur dioxide determination range 1 ppm to 25 ppm)

Sulfuric acid H_2SO_4 [Special class]

Sulfuric acid for readily carbonizable substances To sulfuric acid, the content of which has previously been determined by the following method, add water cautiously, and adjust the concentration to 94.5 to 95.5% of sulfuric acid (H_2SO_4). When the concentration is changed owing to absorption of water during storage, prepare freshly.

Assay: Weigh accurately about 2 g of sulfuric acid in a stopped flask rapidly, add 30 mL of water, cool, and titrate the solution with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of bromothymol blue TS).

$$\text{Each mL of 1 mol/L sodium hydroxide VS} = 49.04 \text{ mg of } H_2SO_4$$

Sulfuric acid TS Cautiously add 1 volume of sulfuric acid to 2 volumes of water, and while warming on a water bath add drop wise potassium permanganate TS, until a pale red color of the solution remains.

Sulfuric acid TS, 0.005 mol/L Dilute 100 mL of 0.05 mol/L sulfuric acid TS with water to make 1000 mL.

Sulfuric acid TS, 0.01 mol/L Dilute 100 mL of 0.1 mol/L sulfuric acid TS with water to make 1000 mL.

Sulfuric acid TS, 0.05 mol/L Dilute 100 mL of 0.5 mol/L sulfuric acid TS with water to make 1000 mL.

Sulfuric acid TS, 0.1 mol/L Add 6 mL of sulfuric acid to 1000 mL of water while slowly shaking, and cool.

Sulfuric acid TS, 0.25 mol/L Add slowly 15 mL of sulfuric acid to 1000 mL of water, while stirring, and cool.

Sulfuric acid TS, 0.5 mol/L Add slowly 30 mL of sulfuric acid to 1000 mL of water, while stirring, and cool.

Sulfuric acid TS, 1 mol/L Add 60 mL of sulfuric acid to 1000 mL of water while slowly shaking, and cool.

Sulfuric acid TS, 2 mol/L Add 120 mL of sulfuric acid to 1000 mL of water while slowly shaking, and cool.

Sulfuric acid TS, for spraying Add 10 mL of sulfuric acid to 90 mL of ethanol (95), and stir carefully and slowly while cooling with ice.

Sulfuric acid-ethanol TS Add slowly 3 mL of sulfuric acid to 1000 mL of ethanol (99.5), while stirring, and cool.

Sulfuric acid-hexane-methanol TS Carefully add 2 mL of sulfuric acid to 230 mL of a mixture of hexane and methanol (1 : 3).

Sulfuric acid-methanol TS Prepare carefully by adding 60 mL of sulfuric acid to 40 mL of methanol.

Sulfuric acid-methanol TS, 0.05 mol/L Add gradually 3 mL of sulfuric acid to 1000 mL of methanol, while stirring, and cool.

Sulfuric acid-sodium dihydrogen phosphate TS Add 6.8 mL of sulfuric acid to 500 mL of water, add 50 g of sodium dihydrogen phosphate dihydrate, dissolve, and add water to make 1000 mL.

Sulfuric acid-sodium hydroxide TS Solution A Add slowly 120 mL of sulfuric acid to 1000 mL of water, while stirring, and cool. Solution B: Dissolve 88.0 g of sodium hydroxide in 1000 mL of freshly boiled and cooled water. Mix equal volumes of solution A and B.

Sulfuric acid, 1.65 mol/L Mix 91 mL of sulfuric acid in water and add water to make 1000 mL..

Sulfuric acid, 25% Take 25 mL of sulfuric acid and add water to make 100.0 mL (Diastase/Protease 500).

Sulfuric acid, dilute Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make 100 mL (10%).

Sulfuric acid, for for readily carbonizable substances See sulfuric acid, for readily carbonizable substances.

Sulfuric acid, fuming $H_2SO_4 \cdot nSO_3$ [Special class]

Sulfuric acid, purified Place sulfuric acid in a beaker, heat until white fumes are evolved, then heat for 3 minutes cautiously and gently. Use after cooling.

Sulfurous acid H_2SO_3 [Special class]

Syntheric zeolite for drying See zeolite, synthetic, for drying.

Synthetic zeolite, for drying See Zeolite, synthetic, for drying

Talc [See monograph, Part II]

Tannic acid [See monograph, Part I]

Tannin acid TS Dissolve 1 g of tannic acid in 1 mL of ethanol and add water to make 10 mL. Prepare before use.

Tartaric acid See L-tartaric acid.

Tartaric acid buffer solution, pH 3.0 Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartrate with 1000 mL of water.

***L*-tartaric acid** $C_4H_6O_6$ [Special Class]

Taurine $C_2H_7NO_3$ White crystals or crystalline powder.
Content: NLT 95%
Assay: Weigh accurately about 0.2 g of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline, dissolve in 50 mL of Water, add 50mL of formaldehyde solution, and titirate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.52 mg $C_2H_7NO_3S$

Tellurium dioxide O_2Te White crystals.
Melting point: About 733 ℃

Terephthalic acid $C_6H_4(COOH)_2$ White crystals or crystalline powder. Slightly soluble in ethanol (95), and practically insoluble in water or ether.
Residue on ignition: NMT 0.3% (1 g).
Content: NLT 95.0%.
Assay: Weigh accurately about 2 g of terephthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide solution to dissolve and titrate with 1 mol/L hydrochloric acid (Indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 83.07 mg $C_8H_6O_4$

Terephthalic acid for gas chromatography Prepared for gas chromatography.

Terphenyl $Na_{18}H_{14}$ A white crystalline powder.
Melting point: 208 ℃ to 213 ℃
Identification: Determine the absorption spectrum as directed under the Ultraviolet-visible Spectroscopy using a solution of Terphenyl in methanol (1 in 250000); it exhibits a maximum absorption at the wavelength between 276 nm and 280 nm.

***P*-Terphenyl** See terphenyl.

Tetra *n*-butylammonium bromide $[CH_3(CH_2)_3]_4NBr$ White crystals or crystalline powder, having a slight, characteristic odor.

Melting point: 101 ℃ to 105 ℃

Purity *Clarity and color of solution*: Dissolve 1.0 g of Tetra n-butylammonium bromide in 20 mL of water; the solution is clear and colorless.

Content: NLT 98.0%.

Assay: Dissolve about 0.5 g of Tetra n-butylammonium bromidee, accurately weighed, in 50 mL of water, add 5 mL of dilute nitric acid, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
32.237 mg of $C_{16}H_{36}NB4$

Tetra n-butylammonium bromide See Tetra *n*-butylammonium bromide.

Tetra n-butylammonium chloride $C_{16}H_{36}ClN$ White color. Deliquescent substance.

Water: NMT 6.0% (0.1 g).

Content: NLT 95.0%, calculated on the anhydrous basis.

Assay: Weigh accurately about 0.25 g of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonicacid, dissolve in 50 mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 27.792 mg of $C_{16}H_{36}$ ClNO

Tetra n-heptylammonium bromide $[CH_3(CH_2)_6]_4NBr$ White crystals or crystalline powder. It has a faint odor.

Melting point: 87 ℃ to 89 ℃

Tetra n-heptylammonium bromide See tetra *n*-heptylammonium bromide.

Tetra n-pentylammonium bromide $[CH_3(CH_2)_4]_4NBr$ White crystals or crystalline powder. It is hygroscopic.

Melting point: 100 ℃ to 101 ℃

Tetra n-pentylammonium bromide See tetra n-pentylammonium bromide.

Tetra-*n*-butylammonium bromide TS, 0.005 mol/L Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate and 1.60 g of tetra-*n*-butylammonium bromide in water to make l000 mL.

Tetra-*n*-butylammonium chloride See tetra *n*-butylammonium chloride.

Tetra-n-butylammonium hydroxide TS, 0.005 mol/L Add 800 mL of water to 13 mL of tetra-*n*-butylammonium hydroxide, adjust the pH to 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. The absorbance at the wavelength of 220 nm using water as the blank is NMT 0.15.

Tetra-n-butylammonium hydroxide TS, 0.4 mol/L Aqueous solution containing tetra-*n*-butylammonium hydroxide at 10 w/v%. Colorless to light yellow, clear liquid, having the odor of ammonia.

Content: 90 to110% of the labeled amount.

Assay: Weigh accurately the mass of a glass-stoppered flask containing 15 mL of water, transfer about 0.2 g of Tetra-n-butylammonium hydroxide, accurately weighed, and titrate with 0.1 mol/L hydrochloric acid VS. (indicator: methyl red TS)

Each mL of 0.1 mol/L hydrochloric acid VS
= 25.948 mg $(C_4H_9)_4NOH$

Tetraammonium cerium(IV) sulfate dihydrate(IV) $Ce(NH_4)_4(SO_4)_4\cdot\cdot 2H_2O$ [Special class]

Tetraammonium cerium(IV) sulfate TS Dissolve 6.8 g of Tetraammonium cerium(IV) sulfate dihydrate in diluted sulfuric acid (3 in 100) to make 100 mL.

Tetraammonium cerium(IV) sulfate(IV)·phosphoric acid TS Dissolve 0.1 g of tetraammonium cerium(IV) sulfate dihydrate in diluted phosphoric acid (4 in 5) to make 100 mL.

Tetrabromophenolphthalein ethyl ester potassium salt $C_{22}H_{13}O_4Br_4K$ [Special Class]

Tetrabromophenolphthalein ethyl ester TS Weigh 0.1 g of tetrabromophenolphthalein ethyl ester potassium salt, add acetic acid (100) to dissolve and make 100 mL. Prepare before use.

Tetrabutylammonium hydrogen sulfate $C_{16}H_{37}NO_4S$ A white crystalline powder. Soluble in alcohol, forming a colorless solution with a slight turbidity.

Melting point: 169 to 173 ℃

Assay: Weigh accurately 170 mg of tetrabutylammonium Hydrogen Sulfate, dissolve in 40 mL of water. Perform a potentiometric titration with 0.1 mol/L sodium hydroxide solution. Perform a blank test in the same way and make necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.95 mg of $C_{16}H_{37}NO_4S$

Tetrabutylammonium hydrogensulfate $C_{16}H_{37}NO_4S$ White crystalline powder. Soluble in alcohol, forming a colorless solution with a slight turbidity.

Melting point: 169 ℃ to 173 ℃

Assay: Weigh accurately 170 mg of Tetrabutylammonium hydrogensulfate and dissolve in 40 mL of water. Proceed with potentiometric titration with 0.1 mol/L sodium hydroxide VS. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium Hydroxide acid VS
= 33.95 mg $C_{16}H_{37}NO_4S$

Tetrabutylammonium hydroxide $(C_4H_9)_4NOH$ [First Class]

Tetrabutylammonium hydroxide TS, 0.005 mol/L To 10 mL of tetrabutylammonium hydroxide TS, add phosphoric acid diluted with 700 mL of water (1 in 10) to adjust the pH to 4.0. Then, add water to make 1000 mL.

Tetrabutylammonium hydroxide TS, 0.005 mol/L Dissolve 40 g of tetrabutylammonium hydroxide in water to make 100 mL. Pipet 3.3 mL of this solution, add 900 mL of water, adjust the pH to 5.0 with 1 mol/L phosphoric acid, and add water to make 1000 mL.

Tetrabutylammonium hydroxide TS, 0.04 mol/L To 100 mL of 0.4 mol/L tetrabutylammonium hydroxide TS, add water to make 1000 mL.

Tetrabutylammonium hydroxide TS, 0.4 mol/L Dissolve 10.38 g of tetrabutylammonium hydroxide in water to make 100 mL.

Tetrabutylammonium hydroxide-methanol TS A methanol solution containing tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$: 259.48] at 25 g/dL. Colorless to light yellow, clear liquid, having the odor of ammonia.

Content: 22.5 to 27.5 g/dL

Assay: Pipet 15 mL of Tetrabutylammonium hydroxide-methanol TS, and titrate with 1 mol/L hydrochloric acid (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS
= 259.48 mg of $C_{16}H_{37}NO$

10% Tetrabutylammonium hydroxide-methanol TS A methanol solution containing tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$: 259.48] at 10 g/100 mL.

Content: 9.0 to 11.0 g/100 mL

Assay: Transfer 2 mL of 10% Tetrabutylammonium hydroxide-methanol TS, accurately measured, to a stoppered flask containing 20 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 25.948 mg of $C_{16}H_{37}NO$

Tetrabutylammonium phosphate $(C_4H_9)_4NH_2PO_4$ White powder. Soluble in water.

Content: NLT 97.0%.

Assay: Dissolve about 1.5 g of Tetrabutylammonium phosphate, accurately weighed, in 80mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide solution VS
= 169.73 $(C_4H_9)_4NH_2PO_4$

Tetrabutylammonium phosphate $(C_4H_9)_4NH_2PO_4$ White powder. Soluble in water.

Content: NLT 97.0%.

Assay: Dissolve about 1.5 g of Tetrabutylammonium phosphate, accurately weighed, in 80mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 169.73 $(C_4H_9)_4NH_2PO_4$

Tetrabutylammonium phosphate solution, 0.005 mol/L, pH 7.0 Dissolve 1.70 g of tetrabutylammonium phosphate in 1 L of water and adjust the pH to 7.0 with ammonia water or phosphoric acid.

4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein sodium $C_{20}H_2C_{14}I_4O_5Na_2$ Scarlet crystals. Freely soluble in water.
Its aqueous solution exhibits a deep red color, and the concentrated sulfuric acid solution exhibits a brown color. Content: NLT 80%.

Tetracycline $C_{22}H_{24}N_2O_8$ Yellow to dark yellow crystals or crystalline powder. Sparingly soluble in ethanol, and practically insoluble in water.

Content: NLT 870 (potency) μg/mg

Assay: Proceed as directed under the Assay of Tetracycline Hydrochloride, and calculate according to the following formula.

$$\text{Potency of tetracycline} = M_s \times \frac{A_T}{A_S} \times 1000$$

M_S: Amount [mg (potency)] of tetracycline hydrochloride RS.

Tetraethylammonium hydroxide TS (10%) $(C_2H_5)_4NOH$ [Tetraethylammonium hydroxide solution(10%), Special Class]

Tetraethylene glycol $C_8H_{18}O_5$ Colorless liquid.

Refractive index n_D^{20}: About 1.46

Boiling point: 177 ℃ to 187 ℃ (1.21 kPa)

Assay: NLT 90%. (Gas/Liquid Chromatography).

Tetrahydrofuran $CH_2(CH_2)_2$ CH $_2$O [Special Class]

Tetrahydroxyquinone $C_6H_4O_6$ Dark blue crystals. The color changes to yellow by light. Soluble in ethanol (95), and sparingly soluble in water.

Tetrahydroxyquinone disodium $C_6H_2O_6Na_2$ [Special Class]

Tetrahydroxyquinone disodium To 1 g of tetrahydroxyquinone disodium, add 300 g of potassium chloride, previously dried for 4 hours at 105 ℃ and cooled in a desiccator, and mix well. Preserve in light-resistant, tight containers.

Tetrahydroxyquinone indicator To 1 g of tetrahydroxyquinone, add 100 g of sucrose and mix evenly.

Tetramethylammonium hydroxide $(CH_3)_4NOH$ Commonly known as an aqueous solution of about 10%. Colorless, clear liquid, having a strong odor of ammonia. Tetramethylammonium hydroxide is more alkaline than ammonia, and readily absorbs carbon dioxide in air. A 10% aqueous solution is used.

Nonvolatile residue: NMT 0.02% (5 mL, 105 ℃, 1 hour).

Purity *Ammonia and other amines*: Weigh accurately about 0.3 g of tetramethylammonium hydroxide into a tared bottle containing about 5 mL of water, and add a slightly excess amount (about 4 mL) of 1 mol/L hydrochloric acid, and evaporate to dryness on a water bath. The amount of tetramethylammonium hydroxide obtained by multiplying 0.8317 by the residue dried for 2 hours at 105 ℃ (tetramethylammonium hydroxide) is ± 0.2% of the amount of tetramethylammonium hydroxide obtained from the Assay.

Content: NLT 90% of the labeled amount.

Assay: Weigh the mass of a glass-stoppered flask containing 15 mL of water, place into about 0.2 g of tetramethylammonium hydroxide, accurately weighed, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: Methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg $(CH_3)_4NOH$

Tetramethylammonium hydroxide TS Add ethanol (99.5) to

15 mL of tetramethylammonium hydroxide, accurately weighed, to make exactly 100 mL.

Tetramethylammonium hydroxide TS pH 5.5 Pipet 15 mL of tetramethylammonium hydroxide, add 990 mL of water and adjust the pH to 5.5 with diluted phosphoric acid (1 in 10)

Tetramethylammonium hydroxide-methanol TS A solution in methanol containing tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15] at 10 g/dL.

Content: 9.0 to 11.0 g/dL

Assay: Pipet 2 mL of Tetramethylammonium hydroxide-methanol TS, to a flask containing 20 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg of $C_4H_{13}NO$

25% Tetramethylammonium hydroxide-methanol TS A solution in methanol containing tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15] at 25 g/100 mL.

Content: 23.0 to 25.0 g/100 mL

Assay: Take exactly about 1 g of this solution, add water to make 50 mL, and titrate with 0.1 mol/L hydrochloric acid (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg of $C_4H_{13}NO$

Tetramethyldiaminodiphenylmethane $C_{17}H_{22}N_2$ 4,4'-methylenebis-(*N*,*N*-Dimethylaniline)
This reagent is white to bluish white foliated crystals, slightly soluble in water, sparingly soluble in alcohol, and soluble in mineral acid.

Melting point: 90 ℃

Tetramethyldiaminodiphenylmethane TS

Solution A: Dissolve 2.5 g of tetramethyldiaminodiphenylmethane in 10 mL of acetic acid (100) and add 50 mL of water to this solution.

Solution B: Dissolve 5 g of potassium iodide in water to make 100 mL.

Solution C: Dissolve 0.3 g of ninhydrin in 10 mL of acetic acid (100) and add 90 mL of water to this solution.

Mix Solution A and Solution B first, and add 1.5 mL of Solution C.

Tetramethylsilane for nuclear magnetic resonance spectroscopy $(CH_3)_4Si$ Prepared for nuclear magnetic resonance spectroscopy.

Theobromine $C_7H_8N_4O_2$ White crystalline solid. Very slightly soluble in water or ethanol, and practically insoluble in benzene, ether or chloroform.

Theophylline $C_7H_8N_4O_2$ White powder. Slightly soluble in water.

Melting point: 269 ℃ to 274 ℃

Purity *Caffeine, theobromine or paraxanthine:* To 0.20 g of Theophylline, add 5 mL of potassium hydroxide TS or 5 mL of ammonia TS; each solution is clear.

Loss on drying: NMT 0.5% (1 g, 105 ℃, 4 hours).

Content: NLT 99.0%.

Assay: Weigh accurately about 0.25 g of theophylline, previously dried, dissolve in 40 mL of *N*,*N*-fimethylformamide, and titrate with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-dimethylformamide TS). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1mol/L sodium methoxide VS
= 18.017 mg $C_7H_8N_4O_2$

Thiamine nitrate $C_{12}H_{17}N_5O_4S$ [See monograph, Part I on Thiamine Nitrate]

Thimerosal $C_9H_9HgNaO_2S$

Thimerosal $CH_3C_6H_3(OH)CH(CH_3)_2$ [See monograph, Part II]

Thimerosal TS (1 in 100) Add purified water to 1.0 g of thimerosal to make 100 mL. Add 250 mL of pH 7.4 phosphate buffer solution (or pH 7.5 imidazole buffer solution) to dissolve, and adjust the pH to 7.5. Add purified water to make 1000 mL. Sterilize it (streptokinase and streptodornase).

Thioacetamide TS To 0.2 mL of 4 w/v% thioacetamide solution, add 1 mL of a mixture of 1 mol/L sodium hydroxide solution, water and 85% glycerin (15 : 5 : 20) , heat on a water bath for 20 seconds, and then cool. Prepare before use.

Thioacetamide TS To 0.2 mL of 4 w/v% thioacetamide solution, add 1 mL of a mixture of 1 mol/L sodium hydroxide solution, water and 85% glycerin (15 : 5 : 20) , heat on a water bath for 20 seconds, cool, and then use immediately (Dried ferrous sulfate-folic acid-cyanocobalamin-ascorbic acid capsule, Hydrotalcite-simethicone tablet, Hydrotalcite-simethicone capsule.

Thioacetamide-glycerin basic TS To 0.2 mL of thioacetamide TS, add 1 mL of alkaline glycerine TS, heat on a water bath for 20 seconds, and then cool. Prepare before use.

Thiodiglycol $S(CH_2CH_2OH)_2$ [β-Thiodiglycol, for automated amino acid analysis] Colorless to pale yellow, clear liquid.

Specific gravity d_{20}^{20}: 1.180 to 1.190

Water: NMT 0.7%.

Thioglycolate medium II for sterility test See the Sterility in the General Tests.

Thioglycolic acid $HSCH_2COOH$ [Mercaptoacetic acid(Thioglycolic acid), Special Class] Preserve in an ampoule and store in a cold, dark place. Not suitable for long-term storage.

THioglycolic acid medium I, for sterility test See the Sterility under the General Tests.

Thioglycolic acid medium II, for sterility test See the Sterility for sterility test See the Sterility Test under the General Tests.

Thioglycollate medium I for sterility test See the Sterility in the General Tests.

Thionyl chloride $SOCl_2$ [Special Class]

Thiosemicarbazide $H_2NCSNHNH_2$ [Special Class]

Thiosemicarbazide $H_2NCSNHNH_2$ [Special Class]

Thiosemicarbazide TS Dissolve 0.1 g of thiosemicarbazide in 50 mL of water and add dilute hydrochloric acid (1 in 2) to make 100 mL. Prepare this test solution immediately prior to use.

Thiourea H_2NCSNH_2 [Special Class]

Thiourea TS To 10 g of thiourea, add water to dissolve and make 100 mL.

***L*-Threonine** C_4H_9NO [See monograph, Part I]

Threoprocaterol hydrochloride $C_{16}H_{22}N_2O_3 \cdot HCl$ Add 10-fold volumes of 3 mol/L hydrochloric acid TS to procaterol hydrochloride and heat for 3 hours under a reflux condenser. After cooling, neutralize (pH 8.5) with sodium hydroxide TS and collect the precipitated crystals. Suspend these crystals in water, add water to adjust the pH to 1 to 2, dissolve, and then add sodium hydroxide to neutralize. Collect the precipitated crystals. Suspend these crystals in 2-propanol and add hydrochloric acid to adjust the pH to 1 to 2. The crystals are dissolved. Then, re-precipitate the crystals. Collect these crystals and air-dry them at 60 ℃. White to pale yellowish white crystals or crystalline powder. It is odorless.

Melting point: About 207 ℃ (with decomposition)

Purity: Dissolve 0.1 g of Threoprocaterol Hydrochloride in 100 mL of a mixture of chloroform and methanol (1 : 1), and use this solution as the test solution. Perform the test with 2 μL of this solution as directed under the Liquid Chromatography according to the operating conditions in the Purity (3) of Procaterol Hydrochloride Hydrate. Determine each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the percentage peak area method: the amount is NLT 95%. For detection sensitivity, add a mixture of water and methanol (1 : 1) to 5.0 mL of the test solution to make 100 mL. Perform the test with 2 μL of this solution. Adjust the sensitivity so that the peak height of threoprocaterol is 5 to 10% of the full scale, and the time span of measurement is about twice the retention time of threoprocaterol after the peak of the solvent.

Thrombin [See monograph, Part II]

Thrombin solution Dissolve 500 units/bottle of thrombin (pharmacopoeia) with 10 mL of 0.1 mol/L phosphate buffer solution (pH 7.2) to make a thrombin solution at 50 units/mL (purified urokinase).

Thrombin TS Immediately prior to use, add 12.5 mL of isotonic sodium chloride injection to 500 units of thrombin (pharmacopoeia) to dissolve. (Prepare before use) (streptokinase and streptodornase).

Thymine $C_5H_6N_2O_2$ White crystalline powder. Slightly soluble in water. Content: NLT 99%. Melting point: 335 ℃ to 337 ℃ (with decomposition)

Thymine-1-naphthol TS Dissolve 0.2 g of thymine in 10 mL of 10 w/v% sodium hydroxide solution and add 10 mL of a solution of 1-naphthol in ethanol (1 in 2500) for blending.

Thymol blue $C_{27}H_{30}O_5S$ [Special Class]

Thymol blue TS Dissolve 100 mg of thymol blue in 100 mL of ethanol (95). Filter, if necessary.

Thymol blue TS See thymol blue TS.

Thymol blue TS, dilute Dissolve 50 mg of thymol blue in 100 mL of ethanol (99.5). Filter, if necessary. Prepare before use.

Thymol blue-dimethylformamide TS Dissolve 100 mg of thymol blue in 100 mL of N,N-dimethylformamide.

Thymol blue-dioxane TS Dissolve 50 mg of thymol blue in 100 mL of 1,4-dioxane. Filter, if necessary. Prepare before use.

Thymolphthalein $C_{28}H_{30}O_4$ [Special Class]

Thymolphthalein TS Dissolve 100 mg of thymolphthalein in 100 mL of ethanol (95). Filter, if necessary.

Thymolphthalein TS See thymolphthalein TS.

Tin See tin.

Tin Sn [Special Class]

Tin(II) chloride dihydrate SnCl2.2H2O [Special Class].

Tin(II) chloride solution To 20 g of tin, add 85 mL of hydrochloric acid, heat it until hydrogen is no longer evolved, and then cool it. Mix 1 mL of this solution with 10 mL of 2 mol/L hydrochloric acid before use.

Tin(II) chloride TS Dissolve 1.5 g of tin(II) chloride dihydrate in 10 mL of water containing a small amount of hydrochloric acid. Preserve in a glass-stoppered bottle in which a fragment of tin has been placed. Use within 1 month.

Tin(II) chloride TS, acidic Dissolve 8 g of tin(II) chloride dihydrate in 500 mL of hydrochloric acid. Preserve in a glass-stoppered bottle. Use within 3 months.

Tin(II) chloride-hydrochloric acid TS To 20 g of tin, add 85 mL of hydrochloric acid, heat it until no more hydrogen gas is evolved, and then cool it. Mix this solution with dilute hydrochloric in the ratio of 1 : 10. Prepare before use.

Tin(II) chloride-sulfuric acid TS Dissolve 10 g of tin(II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

Titan yellow $C_{28}H_{19}N_2Na_2O_6S_2$ [Special Class]

Titanium dioxide See titanium(IV) oxide.

Titanium dioxide TS See titanium(IV) oxide TS.

Titanium trichloride See titanium(III) chloride.

Titanium trichloride solution, 0.2 mol/L Dissolve 30 g of titanium trichloride with 100 mL of hydrochloric acid, and add water to make 1 L (zinc pyrithion).

Titanium trichloride TS See titanium(III) chloride TS

Titanium trichloride-sulfuric acid TS See titanium(III) chloride-sulfuric acid TS.

Titanium(III) chloride TiCl3 [Titanium chloride solution,

First Class] Preserve in a glass-stoppered bottle, protected from the light.

Titanium(III) chloride sulfuric acid TS Mix carefully 20 mL of titanium chloride TS with 13 mL of sulfuric acid, add hydrogen peroxide (30) in small quantities until the color of the solution turns yellow, and heat it until white smoke is evolved. Cool it, and then heat it again by adding water in the same manner. Repeat this process until the solution turns colorless. Then, add water to make 100 mL.

Titanium(III) chloride TS Add dilute hydrochloric acid so that the concentration of titanium(III) chloride becomes 15 g/dL. Prepare before use.

Content: 14.0 g/dL to 16.0 g/dL

Assay: To 2 mL of Titanium(III) chloride TS, add 200 mL of water and 5 mL of hydrochloric acid solution (2 in 3), and while passing carbon dioxide, titrate with 0.1 mol/L ammonium iron(II) sulfate VS (indicator: 5 mL of ammonium thiocyanate TS). The endpoint of the titration is when the solution exhibits a slightly red color.

Each mL of 0.05 mol/L ammonium Iron(III) sulfate VS
= 15.42 mg of TiCl3

Titanium(IV) oxide TiO_2 [Special class].

Titanium(IV) oxide TS To 0.1 g of titanium(IV) oxide, add 100mL of sulfuric acid, and dissolve by gradually heating under direct flame with occasional gentle shaking.

Tocopherol $C_2H_{50}N_2$ [See monograph, Part I]

Tocopherol acetate $C_{31}H_{52}O_3$ [See monograph, Part II on Tocopherol Acetate]

Tocopherol succinate $C_{33}H_{54}O_5$ Immerse 0.5 g of calcium tocopherol succinate in 5 mL of acetic acid (100), add 10 mL of toluene, and warm the mixture for 30 minutes at 70 ℃ with occasional shaking for mixing. After cooling, add 30 mL of water, shake thoroughly to mix and allow the mixture to stand. Discard the water layer, and wash the toluene layer several times with 30 mL each of water until the washings become neutral, and then allow to stand. Add 3 g of anhydrous sodium sulfate to the toluene extract, shake well to mix, take the toluene by decanting, and elute toluene in vacuum; A pale yellow, viscous liquid is obtained. The liquid becomes slightly yellowish solid when stored at ordinary temperature for a long time.

Absorbance $E_{1cm}^{1\%}$ (286 nm) : 38.0 to 42.0 (10 mg, chloroform, 100 mL)

Tolbutamide $C_{12}H_{18}N_2O_3S$ [See monograph, Part I]

***o*-Tolidine** $[-C_6H_3(CH_3)\text{-}4\text{-}NH_2]_2$

***o*-Tolidine TS** Place 160 mg of *o*-tolidine in a 500-mL volumetric flask, add 30 mL of acetic acid (100) to dissolve, add water to make 500 mL, and add 1 g of potassium iodide .

***o*-Tolidine-potassium iodide solution** Dissolve 0.16 g of *o*-tolidine in acetic acid (100) 30 mL and add water to make 500 mL. To this, add potassium iodide 1 g for dissolution. (cyanocobalamin)

***p*-Tolualdehyde** C_8H_8O Colorless to yellow, clear liquid.

Content: NLT 98.0%.

Assay: Perform the test with a solution of p-Tolualdehyde in 5% carbon disulfide solution and perform the test as directed under the Gas Chromatography according to the following conditions.

Operating conditions

Detector: Flame ionization detector

Column: A stainless steel column about 3 mm in internal diameter and about 1.8 m in length, packed with diatomaceous earth for gas chromatography coated with succinic acid diethylene glycol polyester for gas chromatography at 5%.

Sample injection port temperature: 205 ℃

Column temperature: 125 ℃

Carrier gas: Nitrogen

Flow rate: 1.2 mL/min.

Refractive index n_D^{20}: 1.544 to 1.546

***o*-Toluemesulfonamide** $C_7H_9NO_2S$ Colorless crystals or white crystalline powder. Soluble in ethanol (95) and sparingly soluble in water.

Melting point: 157 ℃ to 160 ℃

Purity: *p-Toluenesulfonamide*: Use a solution of *o*-toluemesulfonamide in ethyl acetate (1 in 5000) as the test solution. Perform the test with 10 μL of this solution according to the operating conditions of Purity (6) under Saccharin Sodium as directed under the Gas Chromatography; no peaks other than the peak of *o*-toluemesulfonamide appears. The flow rate is adjusted so that the retention time of *o*-toluemesulfonamide is about 10 minutes. The detection sensitivity is adjusted so that the peak height of *o*-toluemesulfonamide obtained from 10 μL of the test solution is about 50% of the full scale. The time span of peak measurement is about two times the retention time of *o*-toluemesulfonamide after the solvent peak.

Water: NMT 0.5% (4 g, 25 mL of methanol for Karl Fischer titration and 5 mL of pyridine for Karl Fischer titration are used as the solvent.)

Content: NLT 98.5%, calculated on the anhydrous basis.

Assay: Weigh accurately about 25 mg of *o*-toluemesulfonamid and perform the test as directed under the Nitrogen determination.

Each mL of 0.005 mol/L sulfuric acid VS
= 1.7122 mg $C_7H_9NO_2S$

Toluene $C_6H_5CH_3$ [Special Class]

***p*-Toluenesulfonamide** $CH_3C_6H_4SO_2NH_2$ White crystals or crystalline powder.

Melting point: About 137 ℃

Purity *Related substances*: Dissolve 30 mg of p-Toluenesulfonamid in acetone to make exactly 200 mL. Perform the test with 10 μL of this solution as directed in the Purity (3) of Tolazamide; no spot other than the principal spot with the R_f value of 0.6 appears.

***p*-Toluenesulfonic acid monohydrate** $CH_3C_6H_4SO_3H\cdot H_2O$ [Special Class]

***p*-toluenesulfonic acid** See p-Toluenesulfonic acid monohydrate.

***p*-Toluic acid** $CH_3C_6H_4COOH$ White crystalline powder.

Sparingly soluble in warm water. Very soluble in ethanol, methanol or ether.

Content: NLT 98%.

Assay: Weigh about 650 mg of p-toluic acid, dissolve in 25 mL of ethanol, add 25 mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 68.07 $C_8H_8O_2$

Melting point: 181 ℃ to 183 ℃

Toluidine blue $C_{15}H_{16}ClN_3S$ Dark green powder. Soluble in water and slightly soluble in ethanol (95).

***o*-Toluidine hydrochloride solution** Add 1 mL of hydrochloric acid to 0.1 g of *o*-toluidine and add water to make 100 mL (cupric oxide).

***o*-Toluidine** $CH_3C_6H_4NH_2$

Tragacanth powder [See monograph, Part II]

Triamcinolone acetonide $C_{24}H_{31}FO$ [See monograph, Part I]

Tributyrin $C_{15}H_{26}O_6$ Colorless and oily solution. Practically insoluble in water and very soluble in alcohol and ether.

Assay: Perform the test with Tributyrin as directed under the Gas Chromatography according to the following conditions. Calculate the amount of tributyrin; it is NLT 98.0%.

Operating conditions

Detector: Flame ionization detector

Column: A column with about 3 mm in internal diameter and about 1.8 m in length, packed with 80 to 100 μm diatomaceous earth for gas chromatography, coated with succinic acid diethylene glycol polyester for gas chromatography.

Carrier gas: Nitrogen

Sample injection port temperature: 270 ℃

Detector temperature: 300 ℃

1,1,2-Trichloro-1,2,2-trifluoroethane $CFCl_2CF_2Cl$ Colorless and volatile liquid. Miscible with acetone or ether, but not miscible with water.

Purity Related substances: Perform the test with 0.1 μL of 1,1,2-trichloro-1,2,2-trifluoroethane using a micro Syringe for gas chromatography as directed in the Purity (5) Volatile related substances under Halothane; it elutes as a single peak.

Trichloroacetic acid $C_2HCl_3O_2$ [Special Class]

Trichloroacetic acid CCl_3COOH [Special Class]

Trichloroacetic acid solution, 5% Dissolve 5.0 g of trichloroacetic acid in water to make 100 mL.

Trichloroacetic acid TS Dissolve 1.80 g of trichloroacetic acid, 2.99 g of sodium acetate trihydrate and 1.98 g of acetic acid (31) in water to make 100 mL.

Trichloroacetic acid TS, 0.4 mol/L Dissolve 65.4 g of trichloroacetic acid in water to make exactly 1 L.

Trichloroacetic acid TS, 0.4 mol/L Dissolve 65.4 g of trichloroacetic acid TS in water to make 1000 mL (diastase and protease 500).

Trichloroacetic acid TS, pH 7.5 Dissolve 1.8 g of trichloroacetic acid and 1.8 g of anhydrous sodium acetate in 5.5 mL of 6 mol/L acetic acid and water to make 100 mL (serratiopeptidase, semi-alkaline protease).

Trichloroacetic acid-gelatin-tris buffer solution To 1 volume of trichloroacetic acid solution (1 in 5), add 6 volumes of gelatin-tris buffer solution (pH 8.0) and 5 volumes of water.

Triethanolamine See 2,2',2″-Nitrilotriethanol.

Triethylamine $(C_2H_5)_3N$ Colorless liquid with a strong odor of ammonia. Slightly soluble in water, and miscible with ethanol (95) or ether.

Triethylamine-phosphate buffer solution, pH 5.0 Weigh 1.0 mL of triethylamine, add about 900 mL of water to dissolve, adjust the pH to 5.0 with phosphoric acid solution (1 in 10), and add water to make 1000 mL.

Triethylenediamine $C_6H_{12}N_2$ [First Class]

Content: NLT 98%

Trifluoroacetic acid CF_3COOH Clear, colorless liquid, with a strong irritating odor. Freely miscible with water.

Boiling point: 7 ℃ to 73 ℃

Specific gravity d_{20}^{20}: 1.535

Trifluoroacetic acid See trifluoroacetic acid.

0.1% trifluoroacetic acid methanol solution Add methanol to 1 mL of trifluoroacetic acid to make 1000mL.

0.1% trifluoroacetic acid solution Add water to 1 mL of trifluoroacetic acid to make 1000 mL.

Trifluoroacetic acid TS Dissolve 1 mL of trifluoroacetic acid in water to make 1000 mL.

Triketohydrindene hydrate TS See ninhydrin TS.

Trimethylsilyl silica gel for liquid chromatography Prepared for liquid chromatography.

3-(Trimethylsilyl)propanesulfonic acid sodium salt for nuclear magnetic resonance spectroscopy $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$ Prepared for nuclear magnetic resonance spectroscopy.

Trimethylsilylation reagent A mixture of BSA [*N*,*O*-bis (trimethyl-sily) acetamide] and TMCS (trimethyl chlorosilane) (2 : 1)(Prepare before use.)

Trimethylsilylimidazole $C_6H_{12}N_2Si$ A colorless to pale yellow, transparent solution.

Refractive index n_D^{20}: 1.4744 to 1.4764

2,4,6-Trinitrobenzenesulfonic acid dihydrate $C_6H_2(NO_2)_3SO_3H_2 \cdot 2H_2O$ Pale yellow to bright yellow crystals.

Water: 7.0% to 15% (0.1 g, volumetric titration, direct titration).

Content (%) ≥98%

Assay: Weigh accurately 0.3 g of 2,4,6-trinitrobenzenesulfonic acid dihydrate, dissolve in 50 mL of a mixture of water and ethanol (99.5), and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.32 mg $C_6H_2(NO_2)_3SO_3H_2 \cdot H_2O$

2,4,6-Trinitrophenol $HOC_6H_2(NO_2)_3$ [Special Class] Preserve in a well-closed container in a cold place, away from fire.

2,4,6-Trinitrophenol TS, alkaline Mix 20 mL of 2,4,6-trinitrophenol TS with 10 mL of sodium hydroxide solution (1 in 20), and add water to make 100 mL. Use within 2 days after preparation.

2,4,6-Trinitrophenol TS Dissolve 1 g of 2,4,6-trinitrophenol in 100 mL of boiling water and cool it. Filter, if necessary.

2,4,6-Trinitrophenol-ethanol TS Dissolve 1.8 g of 2,4,6-Trinitrophenol in 50 mL of diluted ethanol (99.5) (9 in 10) and 30 mL of water, and add water to make 100 mL.

Trioctylphosphine oxide $C_{24}H_{51}PO$ White crystalline powder. Soluble in organic solvent and practically insoluble in water.

Melting point: between 54 and 56 ℃

Trioctylphosphine oxide See trioctylphosphine oxide.

2,3,5-Triphenyl-2H-tetrazolium hydrochloride $C_{19}H_{15}ClN_4$ [2,3,5-triphenyl-2H-tetrazolium hydrochloride, Special Class]

2,3,5-Triphenyl-2H-tetrazolium hydrochloride TS Dissolve 0.25 g of 2,3,5-triphenyl-2H-tetrazolium hydrochloride in ethanol (95) to make 100 mL. Prepare before use.

Triphenylchloromethane $(C_6H_5)_3CCl$ [Special Class]

Triphenyltetrazolium chloride See 2,3,5-triphenyl-2H-tetrazolium hydrochloride.

Triphenyltetrazolium chloride TS See 2,3,5-triphenyl-2H-tetrazolium hydrochloride TS.

Tris buffer solution Dissolve 60.0 mg of 2-amino-2-hydroxymethyl-1,3-propanediol and 234 mg of sodium chloride in water to make 100 mL (pancreatin).

Tris buffer solution, 0.05 mol/L, pH 7.0 To 6.06 g of 2-Amino-2-hydroxymethyl-1,3-propanediol, add about 750 mL of water to dissolve, adjust the pH to 7.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Tris buffer solution, 0.08 mol/L, pH 8.1 Dissolve 0.294 g of calcium chloride in 40 mL of 2-Amino-2-hydroxymethyl-1,3-propanediol solution, adjust the pH to 8.1 with 1 mol/L hydrochloric acid, and add water to make 100 mL.

Tris buffer solution, 0.1 mol/L, pH 8.0 Dissolve 2.42 g of 2-Amino-2-hydroxymethyl-1,3-propanediol in 100 mL of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

Tris buffer solution, 50 mM, pH 8.0 Add water to 50 mM Tris base (6.055 g) and 8.165 g of 60 mM sodium acetate trihydrate to make 900 mL and dissolve, adjust the pH to 8.0 with hydrochloric acid TS, and add water to make the final volume of 1 L (chondroitin sodium sulfate, chondroitin sodium sulfate capsule).

Tris buffer solution, pH 7.0 Dissolve 24.3 g of 2-Amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid.

Tris buffer solution, pH 8.2 Dissolve 24.2 g of 2-Amino-2-hydroxymethyl-1,3-propanediol in 100 mL of ethylenediaminetetraacetic acid disodium salt TS, add water to make 1 L, and add 1 mol/L hydrochloric acid to adjust the pH to 8.2 (tiopronin).

Tris buffer solution, pH 9.0 Dissolve 36.3 g of tris(hydroxymethyl)aminomethane in 100 mL of water and add 1 mol/L hydrochloric acid to adjust the pH to 9.0 (sodium ferric gluconate complex).

Tris buffer solution, pH 9.5 Dissolve 36.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water and add 1 mol/L hydrochloric acid to adjust the pH to 9.5.

Tris salt buffer solution Dissolve 200 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL.

Tris-hydrochloric acid buffer solution, 0.05 mol/L, pH 7.0 Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 40 mL of 1 mol/L hydrochloric acid TS in about 750 mL of water, adjust the pH to 6.9 to 7.1 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Tris(hydroxymethyl)aminomethane See 2-Amino-2-hydroxymethyl-1,3-propanediol

Tris(hydroxymethyl)aminomethane buffer solution, 0.08 mol/L, pH 8.1 See tris buffer solution, 0.08 mol/L, pH 8.1.

Tris(hydroxymethyl)aminomethane TS Dissolve 1.5 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 100 mL. Pipet 40 mL of this solution and add dimethylsulfoxide to make 200 mL. Use this test solution within 4 hours.

Trisodium citrate dihydrate $C_6H_5Na_3O_7 \cdot 2H_2O$ [Special class or See monograph, Part I on Sodium Citrate]

Trisodium pentacyanoaminoferrate TS See Iron(II) trisodium pentacyanoamine TS.

Trisodium phosphate dodecahydrate $Na_3PO_4 \cdot 12H_2O$ [Special Class]

***(p*-Tritioctylphenoxy)noniethoxyehanol** $C_{34}H_{62}O_{11}$ [First class]

Tropaeolin *OO* solution Add 100 mL of water to 0.1 g of tropaeolin *OO*, warm to dissolve, cool, and filter (loperamide hydrochloride).

Tropaeolin*OO* $NaC_{18}H_{14}SN_3O_3$

Trypsin inhibitor Purified from soybean, and 1 mg of Trypsin inhibitor inhibits 10000 to 30000 BAEE units of trypsin. In

this case, one BAEE unit is defined as the amountt that will inhibit 1 unit of trypsin activity using N alpha-benzoyl L-arginineethyl (BAEE) as a substrate at 25°C, pH 7.6, and liquid volume of 3.2 mL, showing absorbance difference of 0.0001 at 253 nm for 1 minute.

Trypsin inhibitor TS Dissolve 5 mg of trypsin inhibitor in 0.05 mol/L phosphate buffer solution (pH 7.0) to make 10 mL.

Trypsin TS, for elcatonin test To 5 mg of trypsin for liquid chromatography, add 20 mL of ammonium bicarbonate solution (1 in 100) to dissolve. Prepare before use.

L-Tryptophan $C_{11}H_{12}N_2O_2$ [See monograph, Part I]

TTC TS Dissolve 0.8 g of 2,3,5-triphenyl-2H-tetrazolium chloride in water to make 100 mL. Dispense the solution into small test tubes, perform steam sterilization for 15 to 20 minutes at 121 ℃. Then, store away from light.

Tuluidine blue *O* $C_{15}H_{16}ClN_3S$

Tuluidine blue *O* solution, 0.5% Dissolve 0.5 g of tuluidine blue *O* in water to make 100 mL (sodium hyaluronate RS, sodium hyaluronate ocular injection).

Turmeric paper Macerate 20 g of turmeric powder obtained by drying the root of *Curcuma longa* Linné with 100 mL each of cold water 4 times, At each time of maceration, allow to stand and then discard the clear supernatant by decanting, dry the residue at temperatures not higher than 100 ℃, and add 100 mL of ethanol to it, macerate for several days and filter. To the ethanol extract, impregnate a filter paper and air-dry the paper in clean air.

Sensitivity: Dissolve 1 mg of boric acid in a mixture of 1 mL of hydrochloric acid and 4 mL of water, impregnate a sheet of turmeric paper with the length of about 1.5 cm in this solution, take the paper out after 1 minute, and air-dry the paper: the yellow color turns to brown, and when it is moistened with ammonia TS, the color turns again to greenish black.

Turpentine oil [See monograph, Part II]

Twin 20 substrate solution Mix well twin 20.5 mL, 0.025 phenol red TS 1 mL, pH 5.0 acetate buffer solution 10 mL and water 9 mL for mixing. Prepare before use.

***L*-Tyrosine** $C_9H_{11}NO_3$ White crystals or crystalline powder. Odorless and tasteless. Freely soluble in formic acid, very slightly soluble in water, and practically insoluble in ethanol or ether. Dissolves in dilute hydrochloric acid or dilute nitric acid.

Optical rotation $[\alpha]_D^{20}$: -10.5 to -12.5° (After drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm)

Loss on drying: NMT 0.3% (1 g, 105 ℃, 3 hours)

Assay Content: 9.0 to 101.0%. Weigh accurately about 0.3 g of L-tyrosine, previously dried, add exactly 6 mL of formic acid to dissolve, then add exactly 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration under the Titrimetry). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.12 mg $C_9H_{11}NO_3$

Ubiquinone-9 A yellow to orange, crystalline powder. Odorless and tasteless.

Melting point: About 44 ℃

Absorbance $E_{1cm}^{1\%}$ (275 nm): 163 to 190 [ethanol (99.5)]

Uranyl acetate $UO_2(CH_3COO)_2 \cdot 2H_2O$ [Special class]

Uranyl acetate TS Dissolve 1 g of uranyl acetate in water to make 20 mL. Filter, if necessary.

Uranyl acetate-zinc TS Add 5 mL of acetic acid (31) and 50 mL of water to 10 g of Uranyl acetate, heat to dissolve, and use the resulting solution as Solution A. Add 3 mL of acetic acid (31) and 30 mL of water to 30 g of zinc acetate dihydrate, heat to dissolve, and use the resulting solution as Solution B. Mix the two solutions when they are warm, cool the mixture, and then filter it.

Urea H_2NCONH_2 [See monograph, Part II]

Urea See urea.

Urethane $H_2NCOOC_2H_5$ See ethyl carbamate.

***n*-Valeric acid** $CH_3(CH_2)_3COOH$ Clear, colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and ether, and soluble in water.

Boiling point: 186 ℃ and 188 ℃, NLT 89 vol%.

Specific gravity d_4^{20}: 0.936 to 0.942

L-Valine $C_5H_{11}NO_2$ [Special class]

Van urk TS Dissolve 0.2 g of 4-dimethylaminobenzaldehyde in 100 mL of dilute sulfuric acid (65: 35), and add 0.15 mL of 10% ferrous chloride solution (dihydroergocristine mesilate).

Vanadium pentoxide See vanadium(V) oxide.

Vanadium pentoxide TS, dilute See vanadium(V) oxide TS, dilute.

Vanadium(V) oxide Orange to yellowish brown powder.

Identification; Dissolve 0.3 g of vanadium(V) oxide in 10 mL of ammonia TS and 15 mL of water. To 2 mL of this solution, add 20 mL of water, mix, and add 1 mL of copper(II) sulfate TS; yellow precipitates are produced.

Vanadium(V) oxide TS Add vanadium(V) oxide to phosphoric acid, saturate with vanadium(V) oxide by shaking vigorously for 2 hours, and filter through a glass filter.

Vanadium(V) oxide TS, dilute Dissolve 10 mL of dilute vanadium(V) oxide TS with water to make 100 mL. Prepare before use.

Vanillin $C_6H_3CHO(OCH_3)(OH)$ [Special class].

Vanillin-ethanol solution See vanillin-sulfuric acid-ethanol TS.

Vanillin-hydrochloric acid TS Dissolve 5 mg of vanillin with 0.5 mL of ethanol (95), and add 0.5 mL of water and 3 mL of hydrochloric acid. Prepare before use.

Vanillin-sulfuric acid TS Add cautiously 75 mL of sulfuric

acid to 25 mL of ice-cold ethanol (95) After cooling, dissolve with 1 g of vanillin. Prepare before use.

Vanillin-sulfuric acid-ethanol TS Dissolve 3 g of vanillin in ethanol (95) to make 100 mL, and add 0.5 mL of sulfuric acid.

Vasopressin $C_{46}H_{65}N_{15}O_{12}S_2$ A white powder.

Constituent amino acid: Perform the test as directed under the Liquid Chromatography according to the conditions of constituent amino acids of Oxytocin, and determine the respective molar ratios of constituent amino acids with respect to glycine; 0.9 - 1.1 for aspartic acid, 0.9 - 1.1 for glutamic acid, 0.9 - 1.1 for proline, 0.8 - 1.1 for tyrosine, 0.9 - 1.1 for phenylalanine, 0.9 - 1.1 for arginine and 0.8- 1.1 for cystine, and NMT 0.03 for other amino acids.

Vegetable oil [See monograph on Vegetable Oils]

Veratric acid $C_9H_{10}O_4$ [Special class] 3,4-dimethoxy benzoic acid, melting point: about 180 ℃

Vinblastine sulfate $C_{46}H_{58}N_4O_9 \cdot H_2SO_4$ [See monograph, Part I]

Vincristine sulfate $C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$ [See monograph, Part I]

Vinyl acetate $C_4H_6O_2$ A clear, colorless liquid.

Specific gravity: 0.932 to 0.936

Water: NMT 0.2%.

1-Vinyl-2-pyrrolidone C_6H_9NO Clear liquid.

Purity: Perform the test with 0.5 μL of this solution as directed under the Gas Chromatography according to the following conditions. Determine each peak area by the automatic integration method, and determine the amount of 1-vinyl-2-pyrrolidone by the percentage peak area method; it is NLT 99.0%.

Operating conditions

Detector: Flame ionization detector

Column: A glass capillary column about 0.53 mm in internal diameter and about 30 m in length, packed the inner wall with polyethylene glycol 20 M for gas chromatography in a thickness of about 1.0 μm.

Column temperature: Maintain the temperature at 80 °C for 1 minute, then raise at the rate of 10 °C per minute to 190 °C , and hold constant to the temperature for 20 minutes.

Carrier gas: Helium

Column temperature: Constant temperature around 190 ℃

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 15 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 0.5 μL of 1-vinyl-2-pyrrolidone composes about 70% of the full scale.

Time span of measurement: About twice the retention time of 1-vinyl-2-pyrrolidone.

Water: Take 50 mL of methanol for Karl Fischer titration and 10 mL of butyrolactone in a dried titration flask, and titrate with Karl Fischer TS to the endpoint. Weigh accurately 2.5 g of 1-vinyl-2-pyrrolidone, transfer immediately to a titration flask, and perform the test; water is NMT 0.1%.

25% water containing benzoyl peroxide See Benzoylper oxide, 25% water containing.

4% water containing neutral alumina See Neutral alumina, 4% water containing.

Water for bacterial endotoxins test [Water prepared according to the monograph, Pabacterial endotoxins test Water for Injection, or other methods, that does not show reaction at the limit of detection for the lysate reagent used for the bacterial endotoxins test.]

Water for injection [See monograph, Part II]

Water-saturated chloroform Mix chloroform and water at a ratio of 1 : 1, take the chloroform layer, and filter through a glass fiber (prepare before use) (emepronium bromide).

Weakly acidic CM-crosslinked cellulose cation exchanger (type H) Crosslinked, weakly acidic cation exchanger with a heightened strength by introducing the carboxymethyl group with strength to a porous spheric cellulose.

Weakly basic DEAE-crosslinked dextran anion exchanger (type Cl) Weakly basic anion in which the diethylamino ethyl group is introduced to the gel filtration carrier crosslinked dextran.

Wijs TS Place 7.9 g of iodine trichloride and 8.9 g of iodine in separate flasks and add acetic acid (100) in the two flasks to dissolve. Mix the two solutions and add acetic acid (100) again to make 1000 mL. Preserve in a light-resistant glass container.

Xanthhydrol $C_{13}H_{10}O_2$ White to pale yellow powder. Soluble in ethanol (95), diethyl ether, chloroform or acetic acid (100) and practically insoluble in water.

Melting point: 121 to 124 ℃

Residue on ignition: NMT 2.0% (0.5 g).

Xanthhydrol TS Dissolve 150 mg of xanthohydrol in 10 mL of acetic acid (100) and add hydrochloric acid to make 100 mL. Prepare before use.

Xanthon $C_{13}H_8O_2$ Pale yellow powder. Freely soluble in chloroform, and slightly soluble in hot water or ether.

Melting point: 174 to 176 ℃

Purity *Related substances*: Dissolve 50 mg of Xanthone in chloroform to make exactly 10 mL. Perform the test with 5 μL of this solution as directed in the Purity under Propantheline Bromide; no spot other than the principal spot with the R_f value of about 0.7 appears.

Xylene $C_6H_4(CH_3)_2$ [First Class]

Xylene cyanol $C_{25}H_{27}N_2NaO_7S_2$ [Special Class]

***o*-Xylene** $C_6H_4(CH_3)_2$ Colorless, clear liquid.

Specific gravity d_4^{20}: 0.875 to 0.885

Refractive index n_D^{20}: 1.501 to 1.506

Distilling range: 143 to 146 ℃, NLT 95 vol%.

Xylenol orange $C_{31}H_{30}N_2Na_2O_{13}S$ [Special Class]

Xylenol orange TS Dissolve 100 mg of xylenol orange in water to make 100 mL.

Xylitol [See monograph, Part I]

Xylose See D-xylose.

***D*-Xylose** $C_5H_{10}O_5$

Yeast extract A peptone-like substance which represents all the soluble product of yeast cells (Saccharomyces) prepared under optimum conditions, clarified, and dried by evaporating to a powder. One g of yeast extract is derived from NLT 7.5 g of yeast. A reddish yellow to brown powder, having a characteristic but not putrescent odor. Soluble in water, forming a yellow to brown solution, having a slight acidic reaction. It contains no added carbohydrate.

Purity (1) Chloride (calculated as NaCl): NMT 5%.
(2) Coagulable protein: On heating a solution of yeast extract (1 in 20) to boiling, no precipitate is produced.

Loss on drying: NMT 5% (105 °C, constant mass).

Residue on ignition: NMT 15% (0.5 g).

Nitrogen content: Between 7.2% and 9.5% (105 °C constant mass, after drying, Nitrogen Determination).

Yellow beeswax [See monograph, Part II].

Zeolite for gas chromatography (pore diameter 0.5 nm) Prepared for gas chromatography.

Zeolite, synthetic, dry A mixture of $6(Na_2O)\cdot 6(Al_2O_3)\cdot 12(SiO_2)$ and $6(K_2O)\cdot 6(Al_2O_3)\cdot 12(SiO_2)$, prepared for drying. Usually, use the spherically molded form, 2 mm in diameter, prepared by adding a binder. White to grayish white, or color transition by adsorbing water. Average fine pore diameter is about 0.3 mm, and the surface area is 500 to 700 m^2 per g.

Loss on ignition: NMT 2.0% [2 g, 550 to 600°C, 4 hours, desiccator (phosphorus pentoxide)].

Zinc Zn [Special class].

Zinc (standard reagent) Zn [Special class]

Zinc acetate See zinc acetate dihydrate.

Zinc acetate dihydrate $Zn(CH_3COO)_2\cdot 2H_2O$ [Special class]

Zinc chloride $ZnCl_2$ [Special Class]

Zinc chloride TS Dissolve 10 g of zinc chloride and 10 g of potassium hydrogen phthalate in 900mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

Zinc for arsenic assay See zinc, for arsenic analysis.

Zinc iodide-starch paper Impregnate the filter paper for volumetric analysis in freshly prepared zinc iodide-starch TS, and dry in a clean room. Preserve in a glass-stoppered bottle and store away from light and moisture.

Zinc iodide-starch TS To 100 mL of boiling water, add a solution of 0.75 g of potassium iodide in 5 mL of water, a solution of 2 g of zinc chloride in 10 mL of water and a homogeneous suspension of 5 g of starch in 30 mL of water, and mix with stirring. Continue to boil for 2 minutes, and then cool.

Sensitivity: Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitrite solution, 500 mL of water and 10 mL of hydrochloric acid, and touch this solution with the glass rod: an apparent, blue color develops. Place a stopper and store in a cold place.

Zinc powder Zn [Special class]

Zinc sulfate See zinc sulfate heptahydrate.

Zinc sulfate for volumetric analysis See zinc sulfate heptahydrate.

Zinc sulfate for volumetric analysis See zinc sulfate, for volumetric analysis.

Zinc sulfate heptahydrate $ZnSO_4\cdot 7H_2O$ [Special class]

Zinc sulfate TS Dissolve 10 g of zinc sulfate heptahydrate in water to make 100 mL.

Zinc sulfate TS, pH 3.8 Dissolve 125 g of zinc sulfate heptahydrate in water to make 1 L, filter, and adjust pH to 3.8 with 0.1 mol/L hydrochloric acid TS.

Zinc, arsenic-free See zinc for arsenic assay.

Zinc, for arsenic analysis Use Zn with the particle size of 800 μm.

Zincon $C_{20}H_{16}N_4O_6S$ 1-(2-hydroxy-5-sulfophenyl)-3-phenyl-5-(2-carboxyphenyl)formazan Reddish purple powder. Soluble in sodium hydroxide TS, forming a red solution.

Melting point: 210 to 220 °C

Zincon TS Dissolve 0.1 g of zincon in 2mL of 1 mol/L sodium hydroxide solution, and add water to make 100 mL.

Zirconyl nitrate See zirconyl nitrate dihydrate.

Zirconyl nitrate dihydrate $ZrO(NO_3)_2\cdot 2H_2O$ [Special Class]

Zirconyl nitrate TS Dissolve 0.1 g of zirconyl nitrate dihydrate in a mixture of 60 mL of hydrochloric acid and 40 mL of water.

Zirconyl nitrate TS Dissolve 0.1 g of zirconyl nitrate in a mixture of 60 mL of hydrochloric acid and 40 mL of water.

Zirconyl-alizarin S TS Dissolve 0.2 g of zirconyl nitrate dihydrate in 5 mL of dilute hydrochloric acid, add 10 mL of alizarin S TS, and add water to make 30 mL.

3) Standard Solutions for Volumetric Analysis

Standard solutions for volumetric analysis are prepared to a molar concentration specified in the Korean Pharmacopoeia (KP). A 1 molar solution of the standard solution refers to a solution containing exactly 1 mol of the specified substance in 1000 mL and is indicated as 1 mol/L. Standard solutions may be diluted to a specific concentration if necessary. For example, a 1 mol/L solution diluted to 10 times its volume is a 0.1 mol/L solution.
Standard solutions for volumetric analysis should be stored in colorless or light-resistant, stoppered bottles unless otherwise specified.

Preparation and Standardization Standard solutions for volumetric analysis are prepared by one of the following methods. The difference between the actual concentration and the specified concentration *n* (mol/L) is called the normality factor *f*. In the KP, standard solutions are prepared with a normality factor *f* of 0.970 to 1.030. The procedure that determines the normality factor of the prepared standard solution is called standardization.

1) Weigh accurately an amount equivalent to about 1 mole of the pure substance or a multiple or fraction thereof, dissolve in the specified solvent to make exactly 1000 mL, and prepare a standard solution with a concentration close to the specified concentration *n* (mol/L). Divide the amount (g) of the pure substance weighed by the amount (g) of 1 mole of that substance, then divide by the number *n*, which is the specified molar concentration. Use the result as the normality factor *f* of the standard solution. If pure substances are not available, using substances with a high purity whose exact purity is known is allowed.

2) If it is not possible to obtain a pure substance or a highly pure substance whose purity is exactly known, weigh about 1 mole of the pure substance or a multiple or fraction thereof and dissolve it in the specified solvent to make about 1000 mL to prepare a standard solution close to the specified concentration *n* (mol/L). Calculate the normality factor *f* of the standard solution by standardization to determine the exact concentration of this standard solution. There are two methods for standardization: direct and indirect.

a) Direct method: Weigh accurately the specified amount of the specified substance for a standard solution, such as a standard reagent, dissolve it in the specified solvent, and then titrate the resulting solution with the prepared standard solution to obtain the normality factor *f* of the standard solution according to the following equation.

$$f = \frac{1000m}{VMn}$$

M: Amount (g) of substance used to prepare the standard solution (e.g., hydrochloric acid if it is 1 mol/L hydrochloric acid), i.e., the amount of standard reagent corresponding to 1 mol

m: Amount (g) of standard reagent etc., used

V: Amount (mL) of the prepared standard solution consumed in titration

n: A numerical value indicating the specified molar concentration of the prepared standard solution (e.g., if the standard solution has a concentration of 0.02 mol/L, *n* = 0.02)

b) Indirect method: In the case of not using standard reagents directly, take a certain amount V_2 (mL) of the prepared standard solution, titrate with a standard solution for titration whose normality factor (f_1) is known, and calculate the normality factor (f_2) of the prepared standard solution according to the following equation.

$$f_2 = \frac{V_1 \times f_1}{V_2}$$

f_1: Normality factor of the standard solution for titration

f_2: Normality factor of the prepared standard solution

V_1: Amount (mL) of standard solution consume in titration

V_2: Amount (mL) of the prepared standard solution taken

3) Pipet a certain volume of a standard solution whose normality factor is known and dilute it exactly according to the specified method to prepare a standard solution with the specified concentration *n* (mol/L). In this case, the normality factor of the original standard solution and the normality factor of the diluted standard solution are the same.

0.1 mol/L Ammonium ferric sulfate

See 0.1 mol/L ammonium iron(III) sulfate.

0.1 mol/L Ammonium ferric sulfate

See 0.1 mol/L ammonium iron(III) sulfate.

0.02 mol/L Ammonium ferrous sulfate

See 0.02 mol/L ammonium iron(II) sulfate.

0.1 mol/L Ammonium iron(II) sulfate

1000 mL of 0.1 mol/L ammonium iron(II) sulfate contains 39.214 g of ammonium iron(II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$: 392.14].

Preparation Dissolve 40 g of ammonium iron(II) sulfate hexahydrate in a cooled mixture of 30 mL of sulfuric acid and 300 mL of water. Add water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared ammonium iron(II) sulfate solution, add 25 mL of water and 5 mL of phosphoric acid. Titrate the resulting solution with 0.02 mol/L potassium permanganate VS and determine the normality factor.

0.02 mol/L Ammonium iron(II) sulfate

1000 mL of 0.02 mol/L ammonium iron(II) sulfate contains 7.843 g of ammonium iron(II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$: 392.14].

Preparation Add water to 0.1 mol/L ammonium iron(II) sulfate before use to make it exactly 5 times its volume.

0.1 mol/L Ammonium iron(III) sulfate

1000 mL of 0.1 mol/L ammonium iron(III) sulfate contains 48.22 g of ammonium iron(III) sulfate dodecahydrate [$FeNH_4(SO_4)_2 \cdot 12H_2O$: 482.19].

Preparation Dissolve 49 g of ammonium iron(III) sulfate dodecahydrate in a cooled mixture of 6 mL of sulfuric acid and 300 mL of water. Add water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared ammonium iron(III) sulfate solution into an iodine bottle, add 5 mL of hydrochloric acid, shake to mix, and add 2 g of potassium iodide. Stopper the bottle, and allow it to stand for 10 minutes. Then, add 50 mL of water, titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

Note: Store away from light. After prolonged storage, perform standardization before use.

0.1 mol/L Ammonium thiocyanate

1000 mL of 0.1 mol/L ammonium thiocyanate contains 7.612 g of ammonium thiocyanate (NH_4SCN: 76.12).

Preparation Dissolve 8 g of ammonium thiocyanate in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of 0.1 mol/L silver nitrate, and add 50 mL of water, 2 mL of nitric acid, and 2 mL of ammonium iron(III) sulfate TS. Titrate the resulting solution with the prepared ammonium thiocyanate VS until the solution exhibits a persistent reddish brown color while shaking and determine the normality factor.

Note: Store away from light.

0.02 mol/L Ammonium thiocyanate

1000 mL of 0.02 mol/L ammonium thiocyanate contains 1.5224 g of ammonium thiocyanate (NH_4SCN: 76.12).

Preparation Add water to 0.1 mol/L ammonium thiocyanate before use to make it exactly 5 times its volume.

0.1 mol/L Barium chloride

1000 mL of 0.1 mol/L barium chloride contains 24.426 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$: 244.26).

Preparation Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 20 mL of the prepared barium chloride solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter this solution and wash the residue on the filter paper until there is no turbidity when adding silver nitrate TS to the filtrate.

Then, put the washed residue together with the filter paper in a crucible and ignite it to incinerate. Allow it to cool, then add 2 drops of sulfuric acid and ignite again at 700 ℃ for 2 hours. After cooling, weigh accurately the mass of the residue, and use it as the amount of barium sulfate ($BaSO_4$) to determine the normality factor.

Each mL of 0.1 mol/L barium chloride VS
= 23.34 mg of $BaSO_4$

0.02 mol/L Barium chloride

1000 mL of 0.02 mol/L barium chloride contains 4.885 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$: 244.26).

Preparation Dissolve 4.9 g of barium chloride dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 100 mL of the prepared barium chloride solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter this solution and wash the residue on the filter paper until there is no turbidity when adding silver nitrate TS to the filtrate. Then, put the washed residue together with the filter paper in a crucible and ignite it to incinerate. After cooling, add 2 drops of sulfuric acid, and heat strongly again at about 700 °C for 2 hours. After cooling, weigh accurately the mass of the residue, and use it as the amount of barium sulfate ($BaSO_4$) to determine the normality factor.

Each mL of 0.02 mol/L barium chloride VS
= 4.668 mg of $BaSO_4$

0.01 mol/L Barium chloride

1000 mL of 0.01 mol/L barium chloride contains 2.4426 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$: 244.26).

Preparation Add water to 0.02 mol/L barium chloride to make it exactly 2 times its volume before use.

0.005 mol/L Barium perchlorate

1000 mL of 0.005 mol/L barium perchlorate contains 1.6812 g of barium perchlorate [$Ba(ClO_4)_2$: 336.23].

Preparation Dissolve 1.7 g of barium perchlorate in 200 mL of water, add 2-propanol to make 1000 mL, and perform standardization as follows.

Standardization Pipet 20 mL of the prepared barium perchlorate solution, add 55 mL of methanol and 0.15 mL of arsenazo III TS. Titrate the resulting solution with 0.005 mol/L sulfuric acid VS until the color of the solution changes from purple to reddish purple and then red. Determine the normality factor.

0.01 mol/L Bismuth nitrate

1000 mL of 0.01 mol/L bismuth nitrate contains 4.851 g of bismuth nitrate pentahydrate [$Bi(NO_3)_3 \cdot 5H_2O$: 485.07).

Preparation Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add water to make 1000 mL, and perform standardization as follows.

Standardization Pipet 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS. Titrate the resulting solution with 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS until the color of solution changes from red to yellow and determine the normality factor.

0.05 mol/L Bromine

1000 mL of 0.05 mol/L bromine contains 7.990 g of bromine (Br: 79.90).

Preparation Dissolve 2.8 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared bromine solution into an iodine bottle, quickly add 120 mL of water and 5 mL of hydrochloric acid, immediately cap the bottle and shake gently to mix. Add 5 mL of potassium iodide TS, cap the bottle, shake gently, and allow it to stand for 5 minutes. Titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

0.1 mol/L Ceric ammonium sulfate

See 0.1 mol/L tetraammonium cerium(IV) sulfate.

0.01 mol/L Ceric ammonium sulfate

See 0.01 mol/L tetraammonium cerium(IV) sulfate.

0.1 mol/L Cerium sulfate

1000 mL of 0.1 mol/L cerium sulfate contains 33.22 g of cerium sulfate [$Ce(SO_4)_2$: 332.24].

Preparation Use commercially available standard solution for volumetric analysis and perform standardization as follows.

Standardization Weigh accurately about 0.2 g of sodium oxalate, previously dried at 105 °C for 2 hours, as a standard reagent, and dissolve it in 75 mL of water. Then, add a mixture of 5 mL of water and 2 mL of sulfuric acid, mix well, add 10 mL of hydrochloric acid, heat the mixture to 70 to 75 ℃, and titrate the resulting solution with 0.1 mol/L cerium sulfate VS until the solution exhibits a pale yellow color.

Each mL of 0.1 mol/L cerium sulfate VS
= 6.700 mg of $Na_2C_2O_4$

0.01 mol/L Docusate sodium

Preparation Weigh 4.446 g of anhydrous docusate sodium, place it in a 1,000-mL volumetric flask, add water to make 1.0 L, and perform standardization as follows.

Standardization Weigh accurately about 0.35 g of the dried cinepazide maleate RS, transfer it to a 100-mL volumetric

flask, add water and dissolve to make 1000 mL, and use the resulting solution as the standard cinepazide maleate solution (prepare for use). Pipet 10.0 mL of the standard solution and transfer it to a 250-mL Erlenmeyer flask with a stopper. Prepare a solution by dissolving 15 mL of water, 75 mL of chloroform, 5 mL of pH 2.8 acetate buffer solution, and 15 mg of p-dimethylaminoazobenzene in 20 mL of chloroform. Prepare another solution by dissolving 15 mg of oracet blue b dissolved in 3 mL of acetic acid (100) and adding chloroform to make 500 mL. Mix these solutions before use and use the mixture as an indicator. Add 5 mL of this indicator and titrate the resulting solution with 0.01 mol/L docusate sodium. Slowly add the first 5 mL of 0.01 mol/L docusate sodium and continue the titration very slowly. Titrate while stirring vigorously each addition of 0.01 mol/L docusate sodium and allow the titration to run for 30 seconds until the color of the indicator changes from green to grayish pink. Perform a blank test in the same manner and make any necessary correction (cinepazide maleate).

Each mL of 0.1 mol/L docusate sodium
= 5.336 mg of $C_{22}H_{31}N_3O_5 \cdot C_4H_4O_4$

0.1 mol/L Ethylenediaminetetraacetic acid disodium salt
1000 mL of 0.1 mol/L ethylenediaminetetraacetic acid disodium salt contains 37.224 g of ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 38 g of ethylenediaminetetraacetic acid disodium salt dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Wash zinc (standard reagent) with dilute hydrochloric acid, then with water and again with acetone, dry at 110 °C for 5 minutes, and cool in a desiccator (silica gel). Weigh accurately about 1.3 g, add 20 mL of dilute hydrochloric acid and 8 drops of bromine TS, and heat the mixture gently to dissolve. Boil the mixture to remove excess bromine and add water to make exactly 200 mL. Pipet 25 mL of this solution, neutralize it by adding sodium hydroxide solution (1 in 50) and add 5 mL of ammonia/ammonium chloride buffer solution (pH 10.7) and 40 mg of eriochrome Black T-sodium chloride indicator. Titrate the resulting solution with the prepared ethylenediaminetetraacetic acid disodium salt VS until the color of the solution changes from reddish purple to bluish purple and determine the normality factor.

Each mL of 0.1 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 6.539 mg of Zn

Note: Store in polyethylene bottles.

0.05 mol/L Ethylenediaminetetraacetic acid disodium salt
1000 mL of 0.05 mol/L ethylenediaminetetraacetic acid disodium salt contains 18.612 g of ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 19 g of ethylenediaminetetraacetic acid disodium salt dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Wash zinc (standard reagent) with dilute hydrochloric acid, then with water and again with acetone, dry at 110 °C for 5 minutes, and cool in a desiccator (silica gel). Weigh accurately about 0.8 g, add 12 mL of dilute hydrochloric acid and 5 drops of bromine TS, and heat the mixture gently to dissolve. Boil the mixture to remove excess bromine and add water to make exactly 200 mL. Pipet 20 mL of this solution, neutralize it by adding sodium hydroxide solution (1 in 50) and add 5 mL of ammonia/ammonium chloride buffer solution pH 10.7) and 40 mg of eriochrome Black T-sodium chloride indicator. Titrate the resulting solution with the prepared ethylenediaminetetraacetic acid disodium salt VS until the color of the solution changes from reddish purple to bluish purple and determine the normality factor.

Each mL of 0.05 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 3.2695 mg of Zn

Note: Store in polyethylene bottles.

0.02 mol/L Ethylenediaminetetraacetic acid disodium salt
1000 mL of 0.02 mol/L ethylenediaminetetraacetic acid disodium salt contains 7.445 g of ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 7.5 g of ethylenediaminetetraacetic acid disodium salt dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 0.05 mol/L ethylenediaminetetraacetic acid disodium salt. However, Wash zinc (standard reagent) with dilute hydrochloric acid, then with water and again with acetone, dry at 110 °C for 5 minutes, and cool in a desiccator (silica gel). Weigh accurately about 0.3 g, add 5 mL of dilute hydrochloric acid and 5 drops of bromine TS, and proceed in the same manner as the above.

Each mL of 0.02 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 1.3078 mg of Zn

Note: Store in polyethylene bottles.

0.01 mol/L Ethylenediaminetetraacetic acid disodium salt
1000 mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt contains 3.7224 g of ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Add water to 0.02 mol/L ethylenediaminetetraacetic acid disodium salt before use to make it exactly 2 times its volume.

0.001 mol/L Ethylenediaminetetraacetic acid disodium salt
1000 mL of 0.001 mol/L ethylenediaminetetraacetic acid disodium salt contains 0.37224 g of ethylenediaminetetraacetic acid disodium salt ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Add water to 0.01 mol/L ethylenediaminetetraacetic acid disodium salt before use to make it exactly 10 times its volume.

2 mol/L Hydrochloric acid
1000 mL of 2 mol/L hydrochloric acid contains 72.92 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 180 mL of hydrochloric acid to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L hydrochloric acid. However, titrate it by accurately weighing about 2.6 g of sodium carbonate (standard reagent) and dissolving it in 100 mL of water.

Each mL of 2 mol/L hydrochloric acid VS
= 105.99 mg of Na_2CO_3

1 mol/L Hydrochloric acid

1000 mL of 1 mol/L hydrochloric acid contains 36.461 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 90 mL of hydrochloric acid to make 1000 mL and perform standardization as follows.

Standardization Heat sodium carbonate (standard reagent) at 500 to 650 ℃ for 40 to 50 minutes, allow to cool in a desiccator (silica gel), then weigh accurately about 1.3 g, dissolve in 50 mL of water, and add 3 drops of methyl red TS. Titrate the resulting solution with the prepared hydrochloric acid VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a persistent orange to orange-red color when carefully boiled, lightly stoppered, and cooled.

Each mL of 1 mol/L hydrochloric acid VS
= 52.99 mg of Na_2CO_3

0.5 mol/L Hydrochloric acid

1000 mL of 0.5 mol/L hydrochloric acid contains 18.230 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 45 mL of hydrochloric acid to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L hydrochloric acid. However, titrate it by accurately weighing about 0.7 g of sodium carbonate (standard reagent) and dissolving it in 50 mL of water.

Each mL of 0.5 mol/L hydrochloric acid VS
= 26.497 mg of Na_2CO_3

0.2 mol/L Hydrochloric acid

1000 mL of 0.2 mol/L hydrochloric acid contains 7.292 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 18 mL of hydrochloric acid to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L hydrochloric acid. However, titrate it by accurately weighing about 0.3 g of sodium carbonate (standard reagent) and dissolving it in 30 mL of water.

Each mL of 0.2 mol/L hydrochloric acid VS
= 10.599 mg of Na_2CO_3

0.1 mol/L Hydrochloric acid

1000 mL of 0.1 mol/L hydrochloric acid contains 3.6461 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 9.0 mL of hydrochloric acid to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L hydrochloric acid. However, titrate it by accurately weighing about 0.15 g of sodium carbonate (standard reagent) and dissolving it in 30 mL of water.

Each mL of 0.1 mol/L hydrochloric acid VS
= 5.299 mg of Na_2CO_3

0.05 mol/L Hydrochloric acid

1000 mL of 0.05 mol/L hydrochloric acid contains 1.8230 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 0.1 mol/L hydrochloric acid before use to make it exactly 2 times its volume.

0.02 mol/L Hydrochloric acid

1000 mL of 0.02 mol/L hydrochloric acid contains 0.7292 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 0.1 mol/L hydrochloric acid to make it exactly 5 times its volume before use.

0.01 mol/L Hydrochloric acid

1000 mL of 0.01 mol/L hydrochloric acid contains 0.36461 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 0.1 mol/L hydrochloric acid to make it exactly 10 times its volume before use.

0.001 mol/L Hydrochloric acid

1000 mL of 0.001 mol/L hydrochloric acid contains 0.036461 g of hydrochloric acid (HCl: 36.461).

Preparation Add water to 0.1 mol/L hydrochloric acid to make it exactly 100 times its volume before use.

0.05 mol/L Iodine

1000 mL of 0.05 mol/L iodine contains 12.690 g of iodine (I: 126.90).

Preparation Add 100 mL of potassium iodide solution (2 in 5) to 13 g of iodine to dissolve it, add 1 mL of dilute hydrochloric acid and water to make 1000 mL, and perform standardization as follows.

Standardization Grind arsenic trioxide (standard reagent) to powder, dry it at 105 °C for 3 to 4 hours, allow to cool in a desiccator (silica gel), then weigh accurately about 0.15 g and add 20 mL of sodium hydroxide solution (1 in 25) to dissolve it while warming, if necessary. To this solution, add 40 mL of water and 2 drops of methyl orange TS, add dilute hydrochloric acid until the solution turns pale red, and then add 2 g of sodium bicarbonate, 50 mL of water, and 3 mL of starch TS. Titrate the resulting solution by slowly adding the prepared iodine solution until the solution exhibits a persistent blue color and determine the normality factor.

Each mL of 0.05 mol/L iodine VS
= 4.946 mg of As_2O_3

Note: Store away from light. After prolonged storage, perform standardization and make any necessary correction.

0.01 mol/L Iodine

1000 mL of 0.01 mol/L iodine contains 2.5381 g of iodine (I: 126.90).

Preparation Add water to 0.05 mol/L iodine before use to make it exactly 5 times its volume.

0.005 mol/L Iodine

1000 mL of 0.005 mol/L iodine contains 1.2690 g of iodine (I: 126.90).

Preparation Add water to 0.05 mol/L iodine before use to make it exactly 10 times its volume.

0.002 mol/L Iodine

1000 mL of 0.002 mol/L iodine contains 0.5076 g of iodine (I: 126.90).

Preparation Add water to 0.05 mol/L iodine before use to make it exactly 25 times its volume.

0.05 mol/L Magnesium chloride

1000 mL of 0.05 mol/L magnesium chloride contains 10.165 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$: 203.30).

Preparation Dissolve 10.2 g of magnesium chloride hexahydrate in freshly boiled and cooled water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared magne-

sium chloride solution and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and 40 mg of eriochrome black T-sodium chloride indicator. Titrate the resulting solution with the 0.05 mol/L disodium ethylenediaminetetraacetate acetate VS and determine the normality factor. However, the endpoint of the titration is when the color of the solution changes from reddish purple to bluish purple.

0.01 mol/L Magnesium chloride

1000 mL of 0.01 mol/L magnesium chloride contains 2.0330 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$: 203.30).

Preparation Add water to 0.05 mol/L magnesium chloride before use to make it exactly 5 times its volume.

0.05 mol/L Mercuric acetate

See 0.05 mol/L mercury(II) acetate.

0.005 mol/L Mercuric acetate

See 0.005 mol/L mercury(II) acetate.

0.05 mol/L Mercury(II) acetate

1000 mL of 0.05 mol/L mercury(II) acetate contains 15.934 g of mercury(II) acetate [$Hg(CH_3COO)_2$: 318.68].

Preparation Dissolve 16 g of mercury(II) acetate in 5 mL of acetic acid (100) and water to make 1000 mL and perform standardization as follows.

Standardization Dry sodium chloride (standard reagent) at 500 to 650 ℃ for 40 to 50 minutes, then allow it to cool in a desiccator (silica gel). Weigh accurately 5.8 g and dissolve it in water to make exactly 1000 mL. Pipet 20 mL of this solution, add 1 drop of bromophenol blue TS and add dilute nitric acid dropwise until the solution turns yellow. Then, add 5 mL of dilute nitric acid, 100 mL of methanol, and 1 mL of diphenylcarbazone TS, shake well, and titrate the resulting solution with the prepared 0.05 mol/L mercury(II) acetate VS until the color of the solution changes from pale yellow to reddish purple, and determine the normality factor.

Each mL of 0.05 mol/L mercury(II) acetate VS
= 5.844 mg of NaCl

0.005 mol/L Mercury(II) acetate

1000 mL of 0.005 mol/L mercury(II) acetate contains 1.5934 g of mercury(II) acetate [$Hg(CH_3COO)_2$: 318.68].

Preparation Dissolve 1.6 g of mercury(II) acetate in 60 mL of diluted nitric acid (1 in 10), add water to make 1000 mL, and perform standardization as follows.

Standardization Dry sodium chloride (standard reagent) at 500 to 650 ℃ for 40 to 50 minutes, then allow it to cool in a desiccator (silica gel). Weigh accurately 0.58 g and dissolve it in water to make exactly 1000 mL. Pipet 20 mL of this solution, add 1 drop of bromophenol blue TS and add dilute nitric acid dropwise until the solution turns yellow. Then, add 5 mL of dilute nitric acid, 100 mL of methanol, and 1 mL of diphenylcarbazone TS, shake well, and titrate the resulting solution with the prepared 0.005 mol/L mercury(II) acetate VS until the color of the solution changes from pale yellow to reddish purple, and determine the normality factor.

Each mL of 0.005 mol/L mercury(II) acetate VS
= 0.5844 mg of NaCl

0.05 mol/L Oxalic acid

1000 mL of 0.05 mol/L oxalic acid contains 6.303 g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$: 126.07).

Preparation Dissolve 6.3 g of oxalic acid dihydrate in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared oxalic acid solution into a 500-mL Erlenmeyer flask, boil for 10 to 15 minutes, cool to 27 ± 3 ℃, and then add 200 mL of diluted sulfuric acid (1 in 20). Quickly add 22 mL of the newly standardized 0.02 mol/L potassium permanganate solution to a burette with gentle shaking and leave it until the red color of the solution disappears and heat to 55 to 60 ℃. Then, continue the titration until the solution exhibits a pale red color for 30 seconds and determine the normality factor. However, carefully add 0.5 to 1 mL dropwise before the endpoint and after the color of the potassium permanganate solution disappears.

Note: Store away from light.

0.005 mol/L Oxalic acid

1000 mL of 0.005 mol/L oxalic acid contains 0.6303 g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$: 126.07).

Preparation Add water to 0.05 mol/L 0.05 mol/L oxalic acid before use to make it exactly 10 times its volume.

0.05 mol/L Perchloric acid

1000 mL of 0.05 mol/L perchloric acid contains 5.023 g of perchloric acid ($HClO_4$:100.46).

Preparation Add acetic acid for non-aqueous titration to 0.1 mol/L perchloric acid before use to make it exactly twice its volume. Take 8.0 mL of acetic acid for non-aqueous titration and quickly measure the moisture (g/dL). If the moisture exceeds 0.03 (g/dL), use a solution prepared by adding [[moisture (g/dL) - 0.03] × 52.2] mL of acetic anhydride per 1000 mL of acetic acid (100) for non-aqueous titration.

0.1 mol/L Perchloric acid

1000 mL of 0.1 mol/L perchloric acid contains 10.046 g of perchloric acid ($HClO_4$:100.46).

Preparation Slowly add 8.7 mL of perchloric acid to 1000 mL of acetic acid (100) while maintaining the temperature at about 20 °C. Allow the solution to stand for about 1 hour, take 3.0 mL of this solution, and quickly measure the water (g/dL) separately (add water when discarding). Keep this solution at about 20 °C and slowly add [[water (g/dL) - 0.03] × 52.2] mL of acetic anhydride while shaking to mix. Allow the mixture to stand for 24 hours and perform standardization as follows.

Standardization Dry potassium hydrogen phthalate (standard reagent) at 105 °C for 4 hours and then cool it in a desiccator (silica gel). Weigh accurately about 0.5 g, dissolve it in 80 mL of acetic acid (100), and add 3 drops of methylrosaniline chloride TS. Titrate the resulting solution with the prepared perchloric acid VS until the solution exhibits a blue color and determine the normality factor. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.422 mg of $KHC_6H_4(COO)_2$

Note: Store away from moisture.

0.05 mol/L Perchloric acid

1000 mL of 0.05 mol/L Perchloric acid contains 5.023 g of Perchloric acid ($HClO_4$:100.46).

Preparation Add Acetic acid (100) for non-aqueous titration to 0.1 mol/L Perchloric acid before use to make it exactly twice its volume. Take 8.0 mL of Acetic acid (100) for non-aqueous titration and quickly measure the moisture (g/dL). If the moisture exceeds 0.03 (g/dL), use a solution prepared by adding

[[moisture (g/dL) - 0.03] × 52.2] mL of Acetic anhydride per 1000 mL of Acetic acid (100) for non-aqueous titration.

0.02 mol/L Perchloric acid

1000 mL of 0.02 mol/L perchloric acid contains 2.0092 g of perchloric acid ($HClO_4$:100.46).

Preparation Add acetic acid for non-aqueous titration to 0.1 mol/L perchloric acid before use to make it exactly 5 times its volume. Take 8.0 mL of acetic acid for non-aqueous titration and quickly measure the moisture (g/dL). If the moisture exceeds 0.03 (g/dL), use a solution prepared by adding [[moisture (g/dL) - 0.03] × 52.2] mL of acetic anhydride per 1000 mL of acetic acid (100) for non-aqueous titration.

0.1 mol/L Perchloric acid-dioxane

1000 mL of 0.1 mol/L perchloric acid-dioxane contains 10.046 g of perchloric acid ($HClO_4$:100.46).

Preparation Add dioxane to 8.5 mL of perchloric acid to make 1000 mL and perform standardization as follows.

Standardization Dry potassium hydrogen phthalate (standard reagent) at 105 °C for 4 hours and then cool it in a desiccator (silica gel). Weigh accurately about 0.5 g, dissolve it in 80 mL of acetic acid (100), and add 3 drops of methylrosaniline chloride TS. Titrate the resulting solution with the prepared perchloric acid-dioxane VS until the solution exhibits a blue color and determine the normality factor. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 20.422 mg of $KHC_6H_4(COO)_2$

Note: Store away from moisture in a cold place.

0.05 mol/L Perchloric acid-dioxane

1000 mL of 0.05 mol/L perchloric acid-dioxane contains 5.023 g of perchloric acid ($HClO_4$:100.46).

Preparation Add 1,4-dioxane to 0.1 mol/L perchloric acid-dioxane before use to make it exactly twice its volume.

0.004 mol/L Perchloric acid-dioxane

1000 mL of 0.004 mol/L perchloric acid-dioxane contains 0.4018 g of perchloric acid ($HClO_4$:100.46).

Preparation Add 1,4-dioxane to 0.1 mol/L perchloric acid-dioxane before use to make it exactly 25 times its volume.

1/60 mol/L Potassium bichromate

See 1/60 mol/L potassium dichromate.

1/60 mol/L Potassium bromate

1000 mL of 1/60 mol/L potassium bromate contains 2.7833 g of potassium bromate ($KBrO_3$: 167.00).

Preparation Dissolve 2.8 g of potassium bromate in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared potassium bromate into an iodine bottle, add 2 g of potassium iodide and 5 mL of dilute sulfuric acid, cap the bottle, and allow it to stand for 5 minutes. Then, add 100 mL of water and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

1/60 mol/L Potassium dichromate

1000 mL of 1/60 mol/L potassium dichromate contains 4.903 g of potassium bichromate ($K_2Cr_2O_7$: 294.18).

Preparation Grind potassium dichromate (standard reagent) to powder, dry at 100 to 110 °C for 3~ 4 hours, then cool in a desiccator (silica gel). Weigh accurately about 4.903 g and dissolve it in water to make exactly 1000 mL and determine the normality factor.

0.1 mol/L Potassium ferricyanide

See 0.1 mol/L potassium hexacyanoferrate(III).

0.05 mol/L Potassium ferricyanide

See 0.05 mol/L potassium hexacyanoferrate(III).

0.1 mol/L Potassium hexacyanoferrate(III)

1000 mL of 0.1 mol/L potassium hexacyanoferrate(III) contains 32.925 g of potassium hexacyanoferrate(III) [$K_3Fe(CN)_6$: 329.25].

Preparation Dissolve 33 g of potassium hexacyanoferrate(III) in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared potassium hexacyanoferrate(III) solution into an iodine flask, add 2 g of potassium iodide and 10 mL of dilute hydrochloric acid, cap the bottle, and allow it to stand for 15 minutes. Then, add 15 mL of zinc sulfate TS and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS to determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

Note: Store away from light. After prolonged storage, perform standardization before use.

0.05 mol/L Potassium hexacyanoferrate(III)

1000 mL of 0.05 mol/L potassium hexacyanoferrate(III) contains 16.462 g of potassium hexacyanoferrate(III) [$K_3Fe(CN)_6$: 329.25].

Preparation Add water to 0.1 mol/L potassium hexacyanoferrate(III) before use to make it exactly 2 times its volume.

1 mol/L Potassium hydroxide

1000 mL of 1 mol/L potassium hydroxide contains 56.11 g of potassium hydroxide (KOH: 56.11).

Preparation Dissolve 65 g of potassium hydroxide in 950 mL of water. Add the freshly prepared saturated barium hydroxide solution dropwise until no precipitate forms. Mix the solution well, cap it, and allow the mixture to stand for 24 hours. Then, filter the clear supernatant by decantation or with a glass filter and perform standardization as follows.

Standardization Dry amidosulfuric acid (standard reagent) in a desiccator (in vacuum, silica gel) for about 48 hours, weigh accurately about 2.5 g, dissolve in 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate the resulting solution with the prepared potassium hydroxide VS, and solution until the solution exhibits a green color and determine the normality factor.

Each mL of 1 mol/L potassium hydroxide VS
= 97.09 mg of $HOSO_2NH_2$

Note: Store in tightly stoppered bottles or in containers with a carbon dioxide-absorbing tube (soda lime). After prolonged storage, perform standardization before use.

0.5 mol/L Potassium hydroxide

1000 mL of 0.5 mol/L potassium hydroxide contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 32 g of potassium hydroxide and proceed as directed under 1 mol/L potassium hydroxide and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L potassium hydroxide. However, titrate it by accurately weighing about 1.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L potassium hydroxide VS
= 48.55 mg of $HOSO_2NH_2$

Note: Store as directed under 1 mol/L potassium hydroxide. After prolonged storage, perform standardization before use.

0.1 mol/L Potassium hydroxide

1000 mL of 0.1 mol/L potassium hydroxide-ethanol contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 6.5 g of potassium hydroxide and proceed as directed under 1 mol/L potassium hydroxide and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L potassium hydroxide. However, titrate it by accurately weighing about 0.25 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L potassium hydroxide VS
= 9.709 mg of $HOSO_2NH_2$

Note: Store as directed under 1 mol/L potassium hydroxide. After prolonged storage, perform standardization before use.

0.5 mol/L Potassium hydroxide-ethanol

1000 mL of 0.5 mol/L potassium hydroxide-ethanol contains 28.055 g of potassium hydroxide (KOH: 56.11).

Preparation Dissolve 35 g of potassium hydroxide in 20 mL of water, add aldehyde-free ethanol to make 1000 mL, stopper, and allow the mixture to stand for 24 hours. Then, quickly decant the clear supernatant and perform standardization as follows.

Standardization Pipet 25 mL of 0.25 mol/L sulfuric acid, add 50 mL of water and 2 drops of phenolphthalein TS. Titrate the resulting solution with the prepared potassium hydroxide-ethanol VS until the solution exhibits a pale red color and determine the normality factor.

Note: Store in light-resistant, tightly stoppered bottles. Perform standardization before use.

0.1 mol/L Potassium hydroxide-ethanol

1000 mL of 0.1 mol/L potassium hydroxide-ethanol contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 7 g of potassium hydroxide and proceed as directed under 0.5 mol/L potassium hydroxide-ethanol and perform standardization as follows.

Standardization Perform the standardization as directed under 0.5 mol/L potassium hydroxide-ethanol. However, titrate it by taking exactly 0.25 mL of 0.05 mol/L sulfuric acid.

Note: Preserve as directed under 0.5 mol/L potassium hydroxide-ethanol. Perform standardization before use.

0.05 mol/L Potassium hydroxide-ethanol

1000 mL of 0.05 mol/L potassium hydroxide-ethanol contains 2.8055 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 3.5 g of potassium hydroxide and proceed as directed under 0.5 mol/L potassium hydroxide-ethanol and perform standardization as follows.

Standardization Perform the standardization as directed under 0.5 mol/L potassium hydroxide-ethanol. However, titrate it by taking exactly 0.25 mL of 0.025 mol/L sulfuric acid.

Note: Preserve as directed under 0.5 mol/L potassium hydroxide-ethanol. Perform standardization before use.

0.1 mol/L Potassium hydroxide-propanol-benzene

1000 mL of 0.1 mol/L potassium hydroxide-propanol-benzene contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Wash 7 g of potassium hydroxide with 50 mL of 1-propanol, add 250 mL of 1-propanol, shake to dissolve, add anhydrous benzene to make 1000 mL, and perform standardization as follows.

Standardization Dry benzoic acid in a desiccator (silica gel) for 3 hours, weigh accurately about 0.26 g, dissolve it in 50 mL of *N*,*N*-dimethylformamide, and add 10 drops of metanil yellow TS. Titrate the resulting solution with the prepared potassium hydroxide-propanol-benzene VS until the solution exhibits a bluish purple color and determine the normality factor.

Each mL of 0.1 mol/L Potassium hydroxide-propanol-benzene VS
= 12.212 mg of C_6H_5COOH

Note: Preserve in light-resistant bottles.

0.05 mol/L Potassium iodate

1000 mL of 0.05 mol/L potassium iodate contains 10.700 g of potassium iodate (KIO_3: 214.00).

Preparation Dry sodium chloride (standard reagent) at 120 to 140 ℃ for 1.5 to 2 hours, then allow it to cool in a desiccator (silica gel). Weigh accurately 10.700 g, dissolve it in water to make exactly 1000 mL, and determine the normality factor.

1/1200 mol/L Potassium iodate

1000 mL of 1/1200 mol/L potassium iodate contains 0.17833 g of potassium iodate (KIO_3: 214.00).

Preparation Dry sodium chloride (standard reagent) at 120 to 140 ℃ for 1.5 to 2 hours, then allow it to cool in a desiccator (silica gel). Weigh accurately 0.17833 g, dissolve it in water to make exactly 1000 mL, and determine the normality factor.

0.02 mol/L Potassium permanganate

1000 mL of 0.02 mol/L potassium permanganate contains 3.1607 g of potassium permanganate ($KMnO_4$: 158.03).

Preparation Dissolve 3.2 g of potassium permanganate in water to make 1000 mL, boil the mixture for 15 minutes, close the container, and allow it to stand for at least 48 hours. Then, filter the mixture through a glass filter and perform standardization as follows.

Standardization Dry sodium oxalate (standard reagent) at 150 to 200 °C for 1 to 1.5 hours and then cool in a desiccator (silica gel). Weigh accurately about 0.3 g into a 500-mL Erlenmeyer flask and dissolve it in 30 mL of water. Add 250 mL of diluted sulfuric acid (1 in 20) and bring the temperature of the solution to 30 to 35 ℃. Quickly add 40 mL of the prepared potassium permanganate solution to the burette while stirring slowly and leave until the red color of the solution disappears. Then, warm the solution to 55 to 60 ℃ and continue the titration until the solution exhibits a pale red for 30 seconds and determine the normality factor. However, carefully add 0.5 to 1 mL drop-

wise before the endpoint and after the color of the potassium permanganate solution disappears.

Each mL of 0.02 mol/L potassium permanganate VS
= 6.700 mg of $Na_2C_2O_4$

Note: Store away from light. After prolonged storage, perform standardization before use.

0.002 mol/L Potassium permanganate

1000 mL of 0.002 mol/L potassium permanganate contains 0.31607 g of potassium permanganate ($KMnO_4$: 158.03).

Preparation Add water to 0.02 mol/L potassium permanganate before use to make it exactly 10 times its volume.

0.1 mol/L Silver nitrate

1000 mL of 0.1 mol/L silver nitrate contains 16.987 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Dissolve 17.0 g of silver nitrate in water to make 1000 mL and perform standardization as follows.

Standardization Dry sodium chloride (standard reagent) at 500 to 650 °C for 40 to 50 minutes and then cool it in a desiccator (silica gel). Weigh accurately about 0.15 g, dissolve it in 50 mL of water, and add 3 drops of fluorescein sodium TS. Titrate the resulting solution with the prepared silver nitrate VS until the color of the solution changes from yellowish green to yellow and then orange. Determine the normality factor.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Note: Store away from light.

0.02 mol/L Silver nitrate

1000 mL of 0.02 mol/L silver nitrate contains 3.3974 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Add water to 0.1 mol/L silver nitrate before use to make it exactly 5 times its volume.

0.01 mol/L Silver nitrate

1000 mL of 0.01 mol/L silver nitrate contains 1.6987 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Add water to 0.1 mol/L silver nitrate before use to make it exactly 10 times its volume.

0.005 mol/L Silver nitrate

1000 mL of 0.005 mol/L silver nitrate contains 0.8494 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Add water to 0.1 mol/L silver nitrate before use to make it exactly 20 times its volume.

0.001 mol/L Silver nitrate

1000 mL of 0.001 mol/L silver nitrate contains 0.16987 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Add water to 0.1 mol/L silver nitrate before use to make it exactly 100 times its volume.

0.1 mol/L Sodium acetate

1000 mL of 0.1 mol/L sodium acetate contains 8.203 g of sodium methoxide (CH_3COONa: 82.03).

Preparation Dissolve 8.20 g of anhydrous sodium acetate in acetic acid (100) to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared sodium acetate solution and add 50 mL of acetic acid (100) and 1 mL of *p*-naphtholbenzeine TS. Titrate the resulting solution with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellowish brown to yellow and then green, and determine the normality factor. Perform a blank test in the same manner and make any necessary correction.

1 mol/L Sodium hydroxide

1000 mL of 1 mol/L sodium hydroxide contains 39.997 g of sodium hydroxide (NaOH: 39.997).

Preparation Dissolve 42 g of sodium hydroxide in 950 mL of water. Add the freshly prepared saturated barium hydroxide solution dropwise until no precipitate forms. Mix the solution well, cap it, and allow the mixture to stand for 24 hours. Then, filter the clear supernatant by decantation or with a glass filter and perform standardization as follows.

Standardization Dry sulfamic acid (standard reagent) in a desiccator (in vacuum, silica gel) for about 48 hours, weigh accurately about 2.5 g, dissolve in 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate the resulting solution with the prepared sodium hydroxide VS until the solution exhibits a green color and determine the normality factor.

Each mL of 1 mol/L sodium hydroxide VS
= 97.09 mg of $HOSO_2NH_2$

Note: Store in tightly stoppered bottles or in containers with a carbon dioxide-absorbing tube (soda lime). After prolonged storage, perform standardization before use.

0.5 mol/L Sodium hydroxide

1000 mL of 0.5 mol/L sodium hydroxide contains 19.999 g of sodium hydroxide (NaOH: 39.997).

Preparation Weigh 22 g of sodium hydroxide and proceed as directed under 1 mol/L sodium hydroxide and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L sodium hydroxide. However, titrate it by accurately weighing about 1.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L sodium hydroxide VS
= 48.55 mg of $HOSO_2NH_2$

Note: Preserve as directed under 1 mol/L sodium hydroxide. After prolonged storage, perform standardization before use.

0.2 mol/L Sodium hydroxide

1000 mL of 0.2 mol/L sodium hydroxide contains 7.999 g of sodium hydroxide (NaOH: 39.997).

Preparation Weigh 9 g of sodium hydroxide and proceed as directed under 1 mol/L sodium hydroxide and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L sodium hydroxide. However, titrate it by accurately weighing about 0.5 g of amidosulfuric acid (standard reagent).

Each mL of 0.2 mol/L sodium hydroxide VS
= 19.419 mg of $HOSO_2NH_2$

Note: Preserve as directed under 1 mol/L sodium hydroxide. After prolonged storage, perform standardization before use.

0.1 mol/L Sodium hydroxide

1000 mL of 0.1 mol/L sodium hydroxide contains 3.9997 g of sodium hydroxide (NaOH: 39.997).

Preparation Weigh 4.5 g of sodium hydroxide and proceed as directed under 1 mol/L sodium hydroxide and perform standardization as follows.

Standardization Perform the standardization as directed under 1 mol/L sodium hydroxide. However, titrate it by accurately weighing about 0.25 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.709 mg of $HOSO_2NH_2$

Note: Preserve as directed under 1 mol/L sodium hydroxide. After prolonged storage, perform standardization before use.

0.05 mol/L Sodium hydroxide

1000 mL of 0.05 mol/L sodium hydroxide contains 1.9999 g of sodium hydroxide (NaOH: 39.997).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium hydroxide before use to make it exactly twice its volume.

0.02 mol/L Sodium hydroxide

1000 mL of 0.02 mol/L sodium hydroxide contains 0.7999 g of sodium hydroxide (NaOH: 39.997).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium hydroxide before use to make it exactly 5 times its volume.

0.01 mol/L Sodium hydroxide

1000 mL of 0.01 mol/L sodium hydroxide contains 0.39997 g of sodium hydroxide (NaOH: 39.997).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium hydroxide before use to make it exactly 10 times its volume.

0.01 mol/L Sodium lauryl sulfate

1000 mL of 0.01 mol/L sodium lauryl sulfate contains 2.8838 g of sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$: 288.38).

Preparation Dissolve 2.9 g of sodium lauryl sulfate in water to make 1000 mL and perform standardization as follows.

Standardization Dry the papaverine hydrochloride RS, weigh accurately about 0.3 g, and dissolve in water to make 100 mL. Pipet 10 mL of this solution into an Erlenmeyer flask with a stopper, add 5 mL each of water and dilute sulfuric acid and 60 mL of dichloromethane. Add 5 to 6 drops of a methyl yellow solution in dichloromethane (1 in 500) as an indicator, shake vigorously to mix, and titrate the resulting solution with the prepared 0.01 mol/L sodium lauryl sulfate VS using a burette with a minimum scale of 0.02 mL. The endpoint of the titration is when the color of the dichloromethane layer changes from yellow to orange-red after the dropwise addition of 0.01 mol/L sodium lauryl sulfate solution, vigorous shaking to mix and allowing to stand for a while. Determine the normality factor.

Each mL of 0.01 mol/L sodium lauryl sulfate VS
= 3.7585 mg of $C_{20}H_{21}NO_4 \cdot HCl$

0.5 mol/L Sodium methoxide

1000 mL of 0.5 mol/L sodium methoxide contains 27.01 g of sodium methoxide (CH_3ONa: 54.02).

Preparation Add 11.5 g of freshly cut sodium metal piece by piece, i.e., when the reaction is complete, add the remaining metal. Attach a condenser to the flask and add 250 mL of anhydrous methanol in small portions from the top of the condenser to prevent the methanol vapor from evaporating. After adding all of the methanol, connect a drying tube to the top of the condenser, cool the solution, transfer the mixture to a 1000-mL volumetric flask, and add anhydrous methanol to make 1000 mL.

Standardization Pipet 20 mL of 1 mol/L hydrochloric acid, transfer it to a 250-mL flask and add 0.25 mL of phenolphthalein TS. Titrate the resulting solution with the prepared sodium methoxide VS until the solution exhibits a pale red color and determine the normality factor.

0.1 mol/L Sodium methoxide

1000 mL of 0.1 mol/L sodium methoxide contains 5.402 g of sodium methoxide (CH_3ONa: 54.02).

Preparation Dissolve 2.5 g of freshly cut sodium metal fragments in small portions in 150 mL of ice-cooled methanol, then add benzene to make 1000 mL and perform standardization as follows.

Standardization Dry benzoic acid in a desiccator (silica gel) for 24 hours, weigh accurately about 0.3 g, dissolve it in 80 mL of *N*,*N*-dimethylformamide, and add 3 drops of thymol blue-dimethylformamide TS. Titrate the resulting solution with the prepared sodium methoxide VS until the solution exhibits a blue color and determine the normality factor. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 12.212 mg of C_6H_5COOH

Note: Store away from moisture in a cold place. Perform standardization whenever before use.

0.1 mol/L Sodium methoxide-1,4-dioxane

1000 mL of 0.1 mol/L sodium methoxide-1,4-dioxane contains 5.402 g of sodium methoxide (CH_3ONa: 54.02).

Preparation Dissolve 2.5 g of freshly cut sodium metal fragments in small portions in 150 mL of ice-cooled methanol, then add 1,4-dioxane to make 1000 mL and perform standardization as follows.

Standardization Dry benzoic acid in a desiccator (silica gel) for 24 hours, weigh accurately about 0.3 g, dissolve it in 80 mL of *N*,*N*-dimethylformamide, and add 3 drops of thymol blue-dimethylformamide TS. Titrate the resulting solution with the prepared sodium methoxide-dioxane VS until the solution exhibits a blue color and determine the normality factor. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide-dioxane VS
= 12.212 mg of C_6H_5COOH

Note: Store away from moisture in a cold place. Perform standardization whenever before use.

0.1 mol/L Sodium methoxide-dioxane

See 0.1 mol/L sodium methoxide-1,4-dioxane.

0.1 mol/L Sodium nitrite

1000 mL of 0.1 mol/L sodium nitrite contains 6.900 g of sodium nitrite ($NaNO_2$: 69.00).

Preparation Dissolve 7.2 g of sodium nitrite in water to make 1000 mL and perform standardization as follows.

Standardization Dry sulfanilamide for diazotization titration at 105 °C for 3 hours, then allowed to cool in a desiccator

(silica gel). Weigh accurately about 0.44 g and add 10 mL of hydrochloric acid, 40 mL of water, and 10 mL of potassium bromide solution (3 in 10), and cool the mixture to below 15 °C. Titrate the resulting solution with the prepared sodium nitrite VS as directed under the potentiometric titration or amperometric titration and determine the normality factor.

Each mL of 0.1 mol/L sodium nitrite VS
= 17.221 mg of $H_2NC_6H_4SO_2NH_2$

Note: Store away from light. After prolonged storage, perform standardization before use.

0.005 mol/L Sodium oxalate

1000 mL of 0.005 mol/L sodium oxalate contains 0.6700 g of sodium oxalate ($Na_2C_2O_4$: 134.00).

Preparation Dry sodium oxalate (standard reagent) at 150 to 200 °C for 2 hours and allow it to cool in a desiccator (silica gel), weigh accurately about 0.6700 g, dissolve it in water to make exactly 1000 mL, and determine the normality factor.

0.02 mol/L Sodium tetraphenylborate

1000 mL of 0.02 mol/L sodium tetraphenylborate contains 6.844 g of sodium tetraphenylborate [$NaB(C_6H_5)_4$: 342.22].

Preparation Dissolve 7.0 g of sodium tetraphenylborate in water to make 1000 mL and perform standardization as follows.

Standardization Weigh 0.5 g of potassium hydrogen phthalate (standard reagent) and dissolve it in 100 mL of water, add 2 mL of acetic acid (31), warm to 50 °C on a water bath, stir, and slowly add 50 mL of the prepared sodium tetraphenylborate solution using a burette. Then, cool rapidly and allow the solution to stand at ordinary temperature for 1 hour. Filter the resulting precipitate through a previously weighed glass filter, wash three times with 5 mL each of potassium tetraphenylborate TS, and dry at 105 °C for 1 hour. Weigh accurately the mass of the residue and use it as the amount of potassium tetraphenylborate [$KB(C_6H_5)_4$: 358.33] to determine the normality factor.

Each mL of 0.02 mol/L sodium tetraphenylborate VS
= 7.167 mg of $KB(C_6H_5)_4$

Note: Prepare before use.

0.02 mol/L Sodium tetraphenylboron

See 0.02 mol/L sodium tetraphenylborate.

0.1 mol/L Sodium thiosulfate

1000 mL of 0.1 mol/L sodium thiosulfate contains 24.819 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Dissolve 25 g of sodium thiosulfate pentahydrate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL, allow to stand for 24 hours, and perform standardization as follows.

Standardization Dry potassium iodate (standard reagent) at 120 to 140 °C for 1.5 to 2 hours, allow to cool in a desiccator (silica gel), then accurately weigh about 0.1 g in an iodine bottle and dissolve in 25 mL of water. Add 2 g of potassium iodide and 10 mL of dilute sulfuric acid, stopper the bottle, and leave for 10 minutes. Then, add 100 mL of water and titrate the generated iodine with the prepared sodium thiosulfate VS to determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.5667 mg of KIO_3

Note: After prolonged storage, perform standardization and make any necessary correction.

0.05 mol/L Sodium thiosulfate

1000 mL of 0.05 mol/L sodium thiosulfate contains 12.409 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium thiosulfate before use to make it exactly 2 times its volume.

0.02 mol/L Sodium thiosulfate

1000 mL of 0.02 mol/L sodium thiosulfate contains 4.964 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium thiosulfate before use to make it exactly 5 times its volume.

0.01 mol/L Sodium thiosulfate

1000 mL of 0.01 mol/L sodium thiosulfate contains 2.4819 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium thiosulfate before use to make it exactly 10 times its volume.

0.005 mol/L Sodium thiosulfate

1000 mL of 0.005 mol/L sodium thiosulfate contains 1.2409 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Add freshly boiled and cooled water to 0.1 mol/L sodium thiosulfate before use to make it exactly 20 times its volume.

0.5 mol/L Sulfuric acid

1000 mL of 0.5 mol/L sulfuric acid contains 49.04 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Slowly add 30 mL of sulfuric acid to 1000 mL of water while stirring, allow to cool, and perform standardization as follows.

Standardization Heat sodium carbonate (standard reagent) at 500 to 650 °C for 40 to 50 minutes, allow to cool in a desiccator (silica gel), weigh accurately about 1.3 g, dissolve in 50 mL of water, and add 3 drops of methyl red TS. Titrate the resulting solution with the prepared sulfuric acid VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a persistent orange to orange-red color when carefully boiled, lightly stoppered, and cooled.

Each mL of 0.5 mol/L sulfuric acid VS
= 52.99 mg of Na_2CO_3

0.25 mol/L Sulfuric acid

1000 mL of 0.25 mol/L sulfuric acid contains 24.520 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Slowly add 15 mL of sulfuric acid to 1000 mL of water while stirring, allow to cool, and perform standardization as follows.

Standardization Perform the standardization as directed under 0.5 mol/L sulfuric acid. However, titrate it by accurately weighing about 0.7 g of sodium carbonate (standard reagent) and dissolving it in 50 mL of water.

Each mL of 0.25 mol/L sulfuric acid VS
= 26.497 mg of Na_2CO_3

0.1 mol/L Sulfuric acid

1000 mL of 0.1 mol/L sulfuric acid contains 9.808 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Slowly add 6 mL of sulfuric acid to 1000 mL of water while stirring, allow to cool, and perform standardization as follows.

Standardization Perform the standardization as directed under 0.05 mol/L sulfuric acid. However, titrate it by accurately weighing about 0.3 g of sodium carbonate (standard reagent) and dissolving it in 50 mL of water.

Each mL of 0.1 mol/L sulfuric acid VS
= 10.599 mg of Na_2CO_3

0.05 mol/L Sulfuric acid

1000 mL of 0.05 mol/L sulfuric acid contains 4.904 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Slowly add 3 mL of sulfuric acid to 1000 mL of water while stirring, allow to cool, and perform standardization as follows.

Standardization Perform the standardization as directed under 0.05 mol/L sulfuric acid. However, titrate it by accurately weighing about 0.15 g of sodium carbonate (standard reagent) and dissolving it in 30 mL of water.

Each mL of 0.05 mol/L sulfuric acid VS
= 5.299 mg of Na_2CO_3

0.025 mol/L Sulfuric acid

1000 mL of 0.025 mol/L sulfuric acid contains 2.4520 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add water to 0.05 mol/L sulfuric acid before use to make it exactly 2 times its volume.

0.01 mol/L Sulfuric acid

1000 mL of 0.01 mol/L sulfuric acid contains 0.9808 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add water to 0.05 mol/L sulfuric acid before use to make it exactly 5 times its volume.

0.005 mol/L Sulfuric acid

1000 mL of 0.005 mol/L sulfuric acid contains 0.4904 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add water to 0.05 mol/L sulfuric acid before use to make it exactly 10 times its volume.

0.0005 mol/L Sulfuric acid

1000 mL of 0.0005 mol/L sulfuric acid contains 0.04904 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add water to 0.05 mol/L sulfuric acid before use to make it exactly 100 times its volume.

0.1 mol/L Tetraammonium cerium(IV) sulfate

1000 mL of 0.1 mol/L tetraammonium cerium(IV) sulfate contains 63.26 g of tetraammonium cerium(IV) sulfate dihydrate [$Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$: 632.55].

Preparation Dissolve 68 g of tetraammonium cerium(IV) sulfate dihydrate in 0.5 mol/L sulfuric acid to make 1000 mL, allow to stand for 24 hours, filter through a glass filter if necessary, and perform standardization as follows.

Standardization Pipet 25 mL of the prepared tetraammonium cerium(IV) sulfate solution into an iodine bottle, add 20 mL of water and 20 mL of dilute sulfuric acid, and add 1 g of potassium iodide. Then, immediately titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS and determine the normality factor. The endpoint of the titration is when the solution exhibits a pale yellow color near the endpoint and the blue color produced by the addition of 3 mL of starch TS is discolored. Perform a blank test in the same manner and make any necessary correction.

Note: Store away from light. After prolonged storage, perform standardization before use.

0.01 mol/L Tetraammonium cerium(IV) sulfate

1000 mL of 0.01 mol/L tetraammonium cerium(IV) sulfate contains 6.326 g of tetraammonium cerium(IV) sulfate dihydrate [$Ce(NH4)_4(SO4)_4 \cdot 2H_2O$: 632.55].

Preparation Add 0.5 mol/L sulfuric acid to 0.1 mol/L tetraammonium cerium(IV) sulfate before use to make it exactly 10 times its volume.

0.2 mol/L Tetramethylammonium hydroxide

1000 mL of 0.2 mol/L Tetramethylammonium hydroxide contains 18.231 g of tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Before use, take an amount of tetramethylammonium hydroxide-methanol TS equivalent to 18.4 g of tetramethylammonium hydroxide, add water to make 1000 mL, and perform standardization as follows.

Standardization Dry benzoic acid in a desiccator (silica gel) for 24 hours, weigh accurately about 0.6 g, dissolve it in 90 mL of *N*,*N*-dimethylformamide, and add 3 drops of thymol blue-dimethylformamide TS. Titrate the resulting solution with the prepared 0.2 mol/L tetramethylammonium hydroxide VS until the solution exhibits a blue color. Perform a blank test in the same manner to make any necessary correction and determine the normality factor.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS
= 24.425 mg of C_6H_5COOH

Note: Store in tightly stoppered bottles. After prolonged storage, perform standardization before use.

0.1 mol/L Tetrabutylammonium hydroxide

1000 mL of 0.1 mol/L tetrabutylammonium hydroxide contains 25.948 g of tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$: 259.48].

Preparation Before use, take an amount of 10% tetrabutylammonium hydroxide-methanol TS equivalent to 26.0 g of tetrabutylammonium hydroxide, add 2-propanol to make 1000 mL, and perform standardization as follows.

Standardization Dry benzoic acid in a desiccator (silica gel) for 24 hours, weigh accurately about 0.3 g, and dissolve it in 50 mL of acetone. Titrate the resulting solution with the prepared 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank test in the same manner and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
= 12.212 mg of C_6H_5COOH

Note: Store in tightly stoppered bottles. After prolonged storage, perform standardization before use.

0.02 mol/L Tetramethylammonium hydroxide

1000 mL of 0.02 mol/L Tetramethylammonium hydroxide contains 1.8231 g of tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Add freshly boiled and cooled water to 0.1 mol/L tetramethylammonium hydroxide before use to make it exactly 5 times its volume.

0.1 mol/L Tetramethylammonium hydroxide-methanol

1000 mL of 0.1 mol/L tetramethylammonium hydroxide-methanol contains 9.115 g of tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Before use, take an amount of tetramethylammonium hydroxide-methanol TS equivalent to 9.2 g of tetramethylammonium hydroxide, add methanol to make 1000 mL, and perform standardization as follows.

Standardization Perform the standardization as directed under 0.1 mol/L tetramethylammonium hydroxide.

Note: Store in tightly stoppered bottles. After prolonged storage, perform standardization again.

0.1 mol/L Titanium chloride

See 0.1 mol/L titanium(III) chloride.

0.1 mol/L Titanium(III) chloride

1000 mL of 0.1 mol/L titanium(III) chloride contains 15.424 g of titanium(III) chloride ($TiCl_3$: 154.24).

Preparation Add 75 mL of hydrochloric acid to 75 mL of titanium(III) chloride and add freshly boiled and cooled water to make 1000 mL. Place the mixture in a light-resistant burette, replace the air with hydrogen, and allow it to stand for 48 hours before use. Before use, perform standardization as follows.

Standardization Weigh 3 g of ammonium iron(II) sulfate hexahydrate and place it in a 500-mL wide-mouthed Erlenmeyer flask. Dissolve it in 50 mL of freshly boiled and cooled water while passing carbon dioxide. Add 25 mL of diluted sulfuric acid (27 in 100) and quickly add exactly 40 mL of 0.02 mol/L potassium permanganate solution while passing carbon dioxide. To the resulting mixutre, add the titanium(III) chloride solution prepared almost to the endpoint, then immediately add 5 g of ammonium thiocyanate and titrate the resulting solution with the titanium(III) chloride VS to determine the normality factor. The endpoint of titration is when the solution becomes colorless. Perform a blank test in the same manner and make any necessary correction.

Note: Store after replacing the air with hydrogen.

0.1 mol/L Zinc

1000 mL of 0.1 mol/L zinc contains 6.539 g of zinc (Zn: 65.39).

Preparation Wash zinc (standard reagent) with dilute hydrochloric acid, then with water and again with acetone, dry at 110 °C for 5 minutes, and cool in a desiccator (silica gel). To 6.539 g of the washed zinc, add 80 mL of dilute hydrochloric acid and 2.5 mL of bromine TS and dissolve under gentle heating. Boil the mixture to remove excess bromine and add water to make exactly 1000 mL.

0.1 mol/L Zinc sulfate

1000 mL of 0.1 mol/L zinc sulfate contains 28.756 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$: 287.56).

Preparation Dissolve 28.8 g of zinc sulfate heptahydrate for volumetric analysis in water to make 1000 mL and perform standardization as follows.

Standardization Pipet 25 mL of the prepared zinc sulfate solution, add 10.7 mL of pH 10.7 ammonia-ammonium chloride buffer solution and 40 mg of eryochrome black T-sodium chloride indicator TS. Titrate the resulting solution with 0.1 mol/L ethylenediaminetetraacetic acid disodium salt VS until the color of solution changes from reddish purple to bluish purple and determine the normality factor.

0.05 mol/L Zinc acetate

1000 mL of 0.05 mol/L zinc acetate contains 10.975 g of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H2O$: 219.51].

Preparation Dissolve 11.1 g of zinc acetate dihydrate by adding 40 mL of water and 4 mL of dilute acetic acid. Add water to make 1000 mL and perform standardization as follows.

Standardization Pipet 20 mL of 0.05 mol/L ethylenediaminetetraacetic acid disodium salt solution and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and 40 mg of eriochrome black T-sodium chloride indicator. Titrate the resulting solution with the prepared zinc acetate VS and determine the normality factor. The endpoint of the titration is when the color of the solution changes from blue to bluish violet.

0.02 mol/L Zinc acetate

1000 mL of 0.02 mol/L zinc acetate contains 4.390 g of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H2O$: 219.50].

Preparation Dissolve 4.43 g of zinc acetate dihydrate by adding 20 mL of water and 2 mL of dilute acetic acid. Add water to make 1000 mL and perform standardization as follows.

Standardization Perform the standardization as directed under 0.05 mol/L zinc acetate. However, titrate it by taking exactly 0.25 mL of 0.02 mol/L ethylenediaminetetraacetic acid disodium salt.

4) Standard Solutions

Aluminum standard stock solution Weigh 1.0 g of aluminum, add 60 mL of diluted hydrochloric acid (1 in 2), and dissolve by heating. After cooling, add water to make 1000 mL. Pipet 10 mL of this solution, add 30 mL of water and 5 mL of acetic acid-ammonium acetate buffer solution (pH 3.0), and adjust the pH to about 3 by adding ammonia TS dropwise. Then, add 0.5 mL of Cu-PAN TS and titrate the resulting mixture with 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS while boiling it. The endpoint of the titrate is when the color of the solution changes from red to yellow and persist for 1 minutes. Perform a blank test in the same manner and make any necessary correction. Each mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS contains 0.26982 mg of Al

Ammonium standard solution Weigh exactly 2.97 g of ammonium chloride, dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution and add distilled water for ammonium test to make exactly 1000 mL. Each mL of the ammonium standard solution contains 0.01 mg of ammonium (NH_4).

Antimony standard solution Dissolve 0.274 g of antimony potassium tartrate in 20 mL of hydrochloric acid and water to make 100 mL. Pipet 10 mL of this solution and add 200 mL of hydrochloric acid and water to make 1000 mL. Pipet 100 mL of this solution, add 300 mL of hydrochloric acid, and dilute with water to make 1000 mL. Prepare before use. Each mL of the antimony standard solution contains 0.001 mg of antimony (Sb).

Arsenic standard solution Pipet 10 mL of the arsenic standard stock solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the arsenic standard solution contains 1 μg of arsenic trioxide

(As_2O_3). Prepare before use and store in a bottle with a stopper.

Arsenic standard solution See the Arsenic in the General Tests.

Arsenic standard stock solution See the Arsenic in the General Tests.

Arsenic standard stock solution Dry the very fine powder of arsenic trioxide at 105 ℃ for 4 hours, weigh exactly 0.100 g, and dissolve in a 5 mL of sodium hydroxide solution (1 in 5). Neutralize this solution by adding dilute sulfuric and add 10 mL of dilute sulfuric acid again. Add freshly boiled and cooled water to make exactly 1000 mL.

Barium chloride standard solution Dissolve 4.3 g of barium chloride in water to make 1 L. With this solution, determine an amount of sodium sulfate (Na_2SO_4) equivalent to 1 mL of this solution by determining the amount of barium according to the gravimetry. Each mL of of the barium chloride standard solution corresponds to 2.9 mg of sodium sulfate.

Borate pH standard solution See the pH Measurement in the General Tests.

Boron standard solution Dry boric acid in a desiccator (silica gel) to a constant mass, weigh exactly 0.286 g, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 1000 mL. Each mL of the boron standard solution contains 0.5 μg of boron (B).

Cadmium standard solution Pipet 10 mL of the cadmium standard stock solution and add diluted nitric acid (1 in 3) to make exactly 1000 mL. Pipet 10 mL of this solution and add diluted nitric acid (1 in 3) to make 100 mL. Prepare before use. Each mL of the cadmium standard solution contains 0.001 mg of cadmium (Cd).

Cadmium standard stock solution Weigh exactly 1.000 g of cadmium crude metal, add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add dilute nitric acid to make exactly 1000 mL.

Calcium hydroxide pH standard solution See the pH Measurement in the General Tests.

Calcium standard solution Weigh exactly 0.250 g of calcium carbonate, add 5 mL of dilute hydrochloric acid and 25 mL of water, and dissolve by heating. After cooling, add water to make exactly 1000 mL. Each mL of the calcium standard solution contains 0.1 mg of calcium (Ca).

Carbonate pH standard solution See the pH measurement of the General Tests.

Copper standard solution Pipet 10 mL of the copper standard stock solutionand dilute with water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.01 mg of copper (Cu).

Copper standard solution Weigh exactly 0.393 g of copper(II) sulfate pentahydrate, place it in a 100-mL volumetric flask, and dissolve in 60 mL of diluted hydrochloric acid (1 in 100). Add 2 to 3 drops of diluted sulfuric acid (1 in 20), shake well to mix, and then add diluted hydrochloric acid (1 in 100) to make 100 mL. Each mL of the copper standard solution contains 1 mg of copper (Cu).

Copper standard stock solution Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

Cyanide standard solution Pipet a volume of the cyanide standard stock solution equivalent to 10 mg of cyanide (CN), add 100 mL of sodium hydroxide TS and water to make exactly 1000 mL. Prepare before use. Each mL of the cyanide standard solution contains 0.01 mg of cyanide (CN).

Cyanide standard stock solution Dissolve 2.5 g of potassium cyanide in water to make exactly 1000 mL. Pipet 10 mL of this solution, add 0.5 mL of 4-dimethylaminobenzylidenerhodanine TS, and titrate the resulting solution with 0.1 mol/L silver nitrate VS. The endpoint of the titration is when the solution exhibits a red color. Each mL of 0.1 mol/L silver nitrate VS = 5.204 mg of CN.

Dithizone standard solution Dissolve 10 mg of dithizone in 1000 mL of chloroform. Store the dithizone standard solution refrigerated in lead-free containers sealed with a glass stopper and protected from light.

Fluorine standard solution See the Oxygen Flask Combustion in the General Tests.

Gold standard solution, for atomic absorption spectroscopy Pipet 25 mL of the gold standard stock solution and add water to make exactly 1000 mL. Prepare before use. Each mL of the gold standard solution, for atomic absorption spectroscopy contains 0.025 mg of gold (Au).

Gold standard solution for atomic absorption spectroscopy See gold standard solution, for atomic absorption spectroscopy.

Gold standard stock solution Weigh exactly 0.209 g of tetrachloroauric(III) acid tetrahydrate, dissolve in 2 mL of aqua regia, heat the mixture on a water bath for 10 minutes, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of the gold standard stock solution contains 1.00 mg of gold (Au).

Iron standard solution Weigh exactly 86.3 mg of ammonium iron(III) sulfate dodecahydrate, dissolve in 100 mL of water, and add 5mL of dilute hydrochloric acid and water to make exactly 1000 mL. Each mL of the iron standard solution contains 0.01 mg of iron (Fe).

Lead standard solution Pipet 10 mL of the lead standard stock solution and add water to make exactly 100 mL. Prepare before use. Each mL of the lead standard solution contains 0.01 mg of lead (Pb).

Lead standard stock solution Weigh exactly 159.8 mg of lead nitrate, dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Prepare and store the lead standard stock solution using glass containers that do not contain soluble lead salts.

Mercury standard solution Dilute the standard mercury stock solution with 0.001% L-cysteine solution to contain 0 to 200 ng of mercury (Hg) per mL.

Mercury standard solution Dissolve 0.135 g of mercury(II) chloride in 100 mL of 10% nitric acid and add water to make 1000 mL. Dilute this solution with 1% nitric acid before use to make it exactly 1000 times its volume and use this solution as the standard solution. Each mL of the mercury standard solution contains 0.1 μg of mercury (Hg).

Mercury standard solution Dry mercury(II) chloride in a desiccator (silica gel) for 6 hours, weigh exactly 13.5 mg, dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Pipet 10 mL of this solution and add 10 mL of dilute nitric acid and water to make exactly 1000 mL. Prepare before use. Each mL of the mercury standard solution contains 0.1 μg of mercury (Hg).

Mercury standard stock solution Dissolve 0.135 g of mercury(II) chloride in 0.001% L-cysteine solution to make 1000 mL. Each mL of the standard mercury stock solution contains 100 μg of mercury (Hg).

Methanol standard solution See the methanol test in the General Tests.

Nickel standard solution Weigh exactly 6.73 g of ammonium nickel(II) sulfate hexahydrate and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution and add water to make exactly 1000 mL. Each mL of the nickel standard solution contains 0.005 mg of nickel (Ni).

Nickel standard solution for atomic absorption spectroscopy See nickel standard solution, for atomic absorption spectroscopy.

Nickel standard solution, for atomic absorption spectroscopy Pipet 10 mL of the nickel standard stock solution and add water to make exactly 1000 mL. Prepare before use. Each mL of the nickel standard solution for atomic absorption spectroscopy contains 0.01 mg of nickel (Ni).

Nickel standard stock solution Weigh exactly 4.48 g of ammonium nickel(II) sulfate hexahydrate and dissolve in water to make exactly 1000 mL.

Nitric acid standard solution Weigh exactly 0.0722 g of potassium nitrate and dissolve in water to make exactly 1000 mL. Each mL of the nitric acid standard solution contains 0.01 mg of nitrogen (N).

Oxalate pH standard solution See the pH measurement of the General Tests.

pH Standard solution, borate See the pH measurement of the General Tests.

pH Standard solution, calcium hydroxide See the pH measurement of the General Tests.

pH Standard solution, carbonate See the pH measurement of the General Tests.

pH Standard solution, hydroxide See the pH measurement of the General Tests.

pH Standard solution, phosphate See the pH measurement of the General Tests.

pH Standard solution, phthalate See the pH measurement of the General Tests before use.

Phosphate pH standard solution See the pH measurement of the General Tests.

Phosphate standard solution Dilute 1 mL of 0.0715% w/v potassium dihydrogen phosphate solution with water before use to 100 mL (5 ppm PO_4).

Phosphoric acid standard solution Dry potassium dihydrogen phosphate in a desiccator (silica gel) to a constant mass, weigh exactly 0.358 g of it, and add 10 mL of diluted sulfuric acid (3 in 10) and water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 100 mL. Each mL of the phosphoric acid standard solution contains 0.025 mg of phosphoric acid (as PO_4).

Potassium standard stock solution Dry potassium chloride at 130 °C for 2 hours, weigh exactly 9.534 g of it, and dissolve in water to make exactly 1000 mL. Each mL of the potassium standard stock solution contains 5.00 mg of potassium (K).

Selenium standard stock solution Weigh exactly 40.0 mg of selenium metal, add 100 mL of dilute nitric acid (1 in 2) to dissolve (with gentle heating if necessary). Add water to make exactly 1000 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL. Each mL of the selenium standard stock solution contains 0.001 mg of selenium (Se).

Silver standard solution for atomic absorption spectroscopy See silver standard solution, for atomic absorption spectroscopy.

Silver standard solution, for atomic absorption spectroscopy Pipet 10 mL of the silver standard stock solution and add water to make exactly 1000 mL. Prepare before use. Each mL of the silver standard solution, for atomic absorption spectroscopy contains 0.01 mg of silver (Ag).

Silver standard stock solution Weigh exactly 1.575 g of silver nitrate dissolve in water to make exactly 1000 mL. Each mL of the silver standard stock solution contains 1.00 mg of silver (Ag).

Sodium dodecylbenzenesulfonate standard solution Weigh exactly 1.000 g of sodium dodecylbenzenesulfonate and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 1000 mL. Each mL of the sodium dodecylbenzenesulfonate contains 0.01 mg of sodium dodecylbenzenesulfonate [$C_{18}H_{29}SO_4Na$].

Sodium standard solution Dry sodium chloride (standard reagent) at 130 °C for 2 hours, weigh exactly 2.542 g and dissolve in water to make exactly 1000 mL. Each mL of the sodium standard solution contains 1.00 mg of sodium (Na).

Standard solution for amylase test Dissolve 25 g of cobalt chloride and 3.84 g of potassium dichromate in 0.01 mol/L hydrochloric acid to make 100 mL.

Standard solution for viscometer calibration [Industrial standard, standard solution for viscometer calibration]

Tin standard solution Weigh exactly 0.250 g of tin (Sn) and

dissolve in 10 mL of sulfuric acid by heating. After cooling, transfer this solution with 400 mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500 mL. Pipet 10 mL of this solution and add diluted hydrochloric acid (1 in 5) to make exactly 1000 mL. Prepare before use. Each mL of the tin standard solution contains 0.005 mg of tin (Sn).

Tyrosine standard solution See the monograph Pancreatin in the General Tests.

Vinyl chloride standard solution Add approximately 190 mL of ethanol for gas chromatography to a 200-mL volumetric flask and place a silicone rubber stopper. While cooling this volumetric flask in a methanol-dry ice bath, inject 0.20 g of the previously liquefied vinyl chloride through the silicone rubber stopper. Again, inject ethanol for gas chromatography previously cooled in a methanol-dry ice bath through the silicone rubber stopper to make 200 mL. Then, pipet 1 mL of the resulting solution and add ethanol for gas chromatography previously cooled in a methanol-dry ice bath to make exactly 200 mL. Pipet 1 mL of the resulting solution and add ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, to make exactly 100 mL, and use this solution as the standard solution. Store in well-closed containers at below - 20 °C.

Water-methanol standard solution See the Water in the General Tests.

Zinc standard solution Pipet 25 mL of the zinc standard stock solution and add water to make exactly 1000 mL. Prepare before use. Each mL of the zinc standard solution contains 0.025 mg of zinc (Zn).

Zinc standard solution for atomic absorption spectroscopy See the Elastomeric Closures for Injection in the General Tests.

Zinc standard stock solution Weigh exactly 1.000 g of zinc (standard reagent), dissolve in 100 mL of water and 5 mL of hydrochloric acid with gentle heating, cool, and add water to make 1000 mL.

5) Matching Fluids for Color

Matching fluids for color are prepared using the following colorimetric stock solutions. The colorimetric stock solutions are prepared according to the following procedures and stored in glass-stoppered bottles.
When comparing the color of the solution with that of the matching fluids for color, transfer both solutions to Nessler tubes and observe them from the side against a white background unless otherwise specified.

Cobalt(II) chloride hexahydrate colorimetric stock solution
Weigh 65 g of cobalt(II) chloride hexahydrate and dissolve in 25 mL of hydrochloric acid and water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 250 mL. Pipet 25 mL of this solution, add 75 mL of water and 50 mg of murexide-sodium chloride indicator, and add diluted ammonia water (28) dropwise until the color of the solution changes from reddish purple to orange, and titrate the resulting solution with 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS. The endpoint of the titration is when the color of the solution changes from yellow to reddish purple after the addition of 0.2 mL of diluted ammonia water (28) (1 in 10) near the endpoint.

Each mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 2.3793 mg of $CoCl_2 \cdot 6H_2O$

Based on the titration result, add diluted hydrochloric acid (1 in 4) to contain 59.5 mg of cobalt chloride(II) hexahydrate ($CoCl_2 \cdot 6H_2O$: 237.93) per mL, and use this solution as the colorimetric stock solution.

Copper(II) sulfate pentahydrate colorimetric stock solution
Weigh 65 g of copper(II) sulfate pentahydrate and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Pipet 10 mL of this solution and add water to make exactly 250 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 2 mL of dilute ammonia water (28) (1 in 10) and 50 mg of murexide-sodium chloride indicator, and titrate the resulting solution with 0.01 mol/L disodium ethylenediaminetetraacetate VS. The endpoint of the titration is when the color of the solution changes from green to violet.

Each mL of 0.01 mol/L ethylenediaminetetraacetic acid disodium salt VS
= 2.4969 mg of $CuSO_4 \cdot 5H_2O$

Based on the titration result, add diluted hydrochloric acid (1 in 4) to contain 62.4 mg of copper sulfate(II) pentahydrate ($CuSO_4 \cdot 5H_2O$: 249.68) per mL, and use this solution as the colorimetric stock solution.

Iron(III) chloride hexahydrate colorimetric stock solution
Weigh 55 g of iron(III) chloride hexahydrate and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Pipet 10 mL of this solution, transfer it to an iodine flask, add 15 mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water to the mixture, and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 27.030 mg of $FeCl_2 \cdot 6H_2O$

Based on the titration result, add diluted hydrochloric acid (1 in 4) to contain 45.0 mg of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$: 270.30) per mL, and use this solution as the colorimetric stock solution.

Matching Fluids for Color
Using a burette or pipette with a scale of 0.1 mL or less, take a given amount of the colorimetric stock solution of each color and water listed in Table 1 and mix well.

Symbol of the matching fluids for color	Iron(III) chloride hexahydrate colorimetric stock solution (mL)	Cobalt(II) chloride hexahydrate colorimetric stock solution (mL)	Copper(II) sulfate pentahydrate colorimetric stock solution (mL)	Water (mL)
A	0.4	0.1	0.1	4.4
B	0.9	0.3	0.3	3.5
C	0.6	0.1	0.1	4.2
D	0.6	0.3	0.4	3.7
E	1.2	0.4	0.3	3.1

F	1.2	0.3	-	3.5
G	1.2	0.5	0.2	3.1
H	1.5	0.2	-	3.3
I	2.2	0.4	0.1	2.3
J	3.5	0.4	0.1	1.0
K	4.5	0.5	-	-
L	3.8	0.8	0.1	0.3
M	2.0	0.1	0.1	2.8
N	4.9	-	0.1	-
O	4.8	0.1	0.1	-
P	0.4	0.2	0.1	4.3
Q	0.3	0.2	0.1	4.4
R	0.4	0.3	0.2	4.1
S	0.1	0.2	-	4.7
T	0.5	0.5	0.4	3.6

6) Optical Filters for Wavelength and Transmission Rate Calibration

For the optical filter for wavelength and transmission rate calibration, use the appropriate one of the following filters considering the wavelength of the instrument to be calibrated. Optical filters for transmission rate calibration are also used for absorbance correction.

Optical Filters for transmission rate calibration
Spectral Transmittance reference material for optical calibration filter C70329-00 provided by Korea Research Institute of Standards and Science

Calibration method: Measure the spectral transmittance at the recorded wavelength using a reference spectrophotometer.

Classification: Low transmittance 10%, medium transmittance 30% to 50%, high transmittance 90%
Measurement range: NLT 10 measurement points from 250 nm to 750 nm, at an interval of 50 nm

Optical Filters for wavelength calibration

1) Optical calibration filter C70326-01 (wavelength calibration filter) provided by Korea Research Institute of Standards and Science

Calibration method: Measure the absorption peaks at different wavelengths within the recorded wavelength range using a reference spectrophotometer.

Measurement range: NLT 10 measurement points from 380 nm to 780 nm

2) Optical calibration filter C70326-02 (wavelength calibration filter) provided by Korea Research Institute of Standards and Science

Calibration method: Measure the absorption peaks at different wavelengths within the recorded wavelength range using a reference spectrophotometer.

Measurement range: NLT 9 measurement points from 240 nm to 640 nm.

7) Measuring Instruments and Vessels

Thermometer Generally, a partial immersion thermometer (rod-shaped) or an immersion mercury thermometer (rod-shaped) is used after determining an instrumental error. However, a partial immersion thermometer (rod-shaped) is used for Congealing Temperature, Melting Point (method 1), Boiling Point and Distilling Range. Table 2.

Chemical volumetric instruments Use flasks, pipettes, burettes, and measuring cylinders conforming to industrial standards.

Cassia flask Cassia flask is a tempered glass flask with a stopper and has a capacity mark on the neck, as shown in Figure 1.

Nessler tube Nessler tube is a colorless, tempered glass cylinder with a thickness of 1.0 to 1.5 mm and has a tempered glass stopper, as shown in Figure 2. However, the height difference between the 50 mL scale bars of the individual tubes should be NMT 2 mm.

Balances and calibration weights (1) Chemical balance Use those capable of weighing up to 0.1 mg.

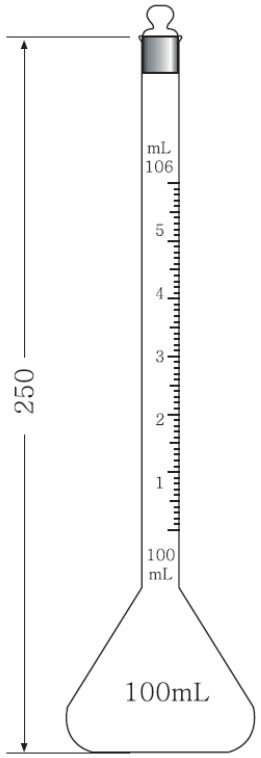
(2) Semi-micro balance Use those capable of weighing up to 0.01 mg.

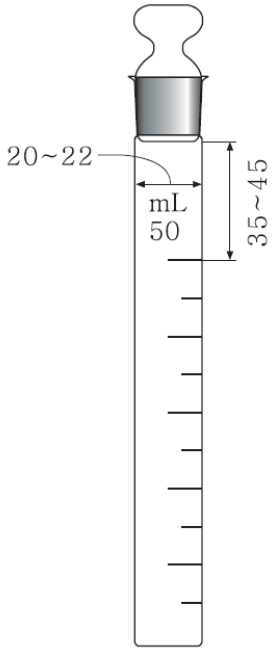
(3) Microbalances Use those capable of weighing up to 0.001 mg.

(4) Calibration weights Use those validated.

Gas mixer Use the apparatus made of tempered glass, as shown in Figure 3.

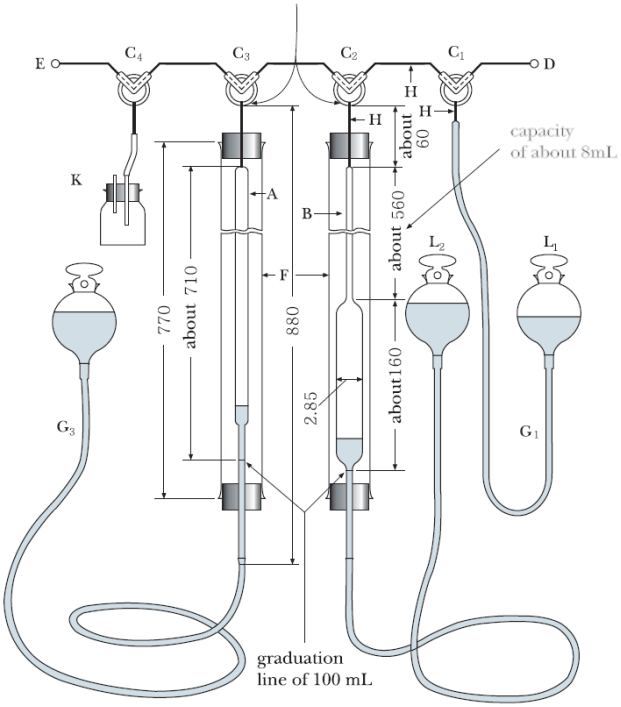
Sieve Use sieves that conform to the specifications in the following Table 3. Each sieve name presents with a sieve number or nominal size (μm).





* The figures are in mm.

Figure 1 **Figure 2**



* The figures are in mm.

Figure 3

A: Gas burette (capacity of 100 mL, internal diameter of about 13.7 mm, 0.2 mL graduation. However, the narrow part on the bottom is a 0.1 mL scale).

B: Gas burette (capacity of 100 mL, the internal diameter of the upper part is about 4.2 mm with a 0.02 mL scale. The internal diameter of the lower part is about 28.5 mm with a 1 mL scale).

C: (C1, C2, C3, and C4): 3-Way stopcocks

D: Entrance for sample collection (bent forward to a length of 20 mm)

E: Mixed gas inlet (bent forward to a length of 20 mm)

F: Jacket tube (about 770 mm in length, 40 mm of outer diameter, almost filled with water at ordinary temperature)

G: Thick-walled rubber hose with an internal diameter of about 4 mm (length of G1 is about 80 cm, G2 and G3 are about 120 cm long)

H: Capillary tube with thick tube wall (internal diameter of about 1 mm)

K: Receiving flask

L: Leveling bulb (L1 is filled with 50 mL of mercury and L2 and L3 with about 150 mL)

Table 2. Partial immersion thermometer

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above the liquid	Nitrogen or argon	Nitrogen or argon	Nitrogen or argon	Nitrogen or argon	Nitrogen or argon	Nitrogen or argon
Temperature range (℃)	-17 to 50	40 to 100	90 to 150	140 to 200	190 to 250	240 to 320
Minimum scale (℃)	0.2	0.2	0.2	0.2	0.2	0.2
Longer major graduations at	Every 1 ℃	Every 1 ℃	Every 1 ℃	Every 1 ℃	Every 1 ℃	Every 1 ℃
Number markings at	Every 2 ℃	Every 2 ℃	Every 2 ℃	Every 2 ℃	Every 2 ℃	Every 2 ℃
Length (mm)	280 to 300	280 to 300	280 to 300	280 to 300	280 to 300	280 to 300
Stem diameter (mm)	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3
Length of mercury bulb (mm)	12 to 18	12 to 18	12 to 18	12 to 18	12 to 18	12 to 18
Distance from the bottom of the mercury bulb to the lowest graduation mark (mm)	75 to 90	75 to 90	75 to 90	75 to 90	75 to 90	75 to 90
Distance from the top of the thermometer to the highest graduation mark (mm)	35 to 65	35 to 65	35 to 65	35 to 65	35 to 65	35 to 65
Distance from the bottom of the mercury bulb to the immersion line (mm)	58 to 62	58 to 62	58 to 62	58 to 62	58 to 62	58 to 62
Tip shape	Ring-shaped	Ring-shaped	Ring-shaped	Ring-shaped	Ring-shaped	Ring-shaped
Test temperature	-15 15 45	45 70 95	95 120 145	145 170 195	195 220 245	245 280 315
Tolerance (℃)	0.2	0.2	0.2	0.2	195 : 0.2 220 : 0.3 245 : 0.3	245 : 0.3 280 : 0.4 315 : 0.5

Table 3. Analytical sieve specifications

Sieve No.	Nominal mesh size (μm)	Analytical sieve specifications					
		Mesh size			Screen wire		
		Dimensions (mm)	Tolerance		Wire diameter (mm)	Wire diameter tolerance	
			Mean (± X)	Maximum (+Y)		Maximum wire diameter	Minimum wire diameter
3.5	5600	5.6	0.18	0.47	1.6	1.9	1.3
4	4750	4.47	0.15	0.41	1.6	1.9	1.3
4.7	4000	4	0.13	0.37	1.4	1.7	1.2
5.5	3350	3.35	0.11	0.32	1.25	1.5	1.06
6.5	2800	2.8	0.09	0.29	1.12	1.3	0.95
7.5	2360	2.36	0.08	0.25	1	1.15	0.85
8.6	2000	2	0.07	0.23	0.9	1.04	0.77
10	1700	1.7	0.06	0.2	0.8	0.92	0.68
12	1400	1.4	0.05	0.18	0.71	0.82	0.6
14	1180	1.18	0.04	0.16	0.63	0.72	0.54
16	1000	1	0.03	0.14	0.56	0.64	0.48
18	850	0.85	0.029	0.127	0.5	0.58	0.43
22	710	0.71	0.025	0.112	0.45	0.52	0.38
26	600	0.6	0.021	0.101	0.4	0.46	0.34
30	500	0.5	0.018	0.089	0.315	0.36	0.27
36	425	0.425	0.016	0.081	0.280	0.32	0.24
42	355	0.355	0.013	0.072	0.224	0.26	0.19
50	300	0.3	0.012	0.065	0.200	0.23	0.17
60	250	0.25	0.0099	0.058	0.16	0.19	0.13
70	212	0.212	0.0087	0.052	0.14	0.17	0.12
83	180	0.18	0.0076	0.047	0.125	0.15	0.106
100	150	0.15	0.0066	0.043	0.1	0.115	0.085
119	125	0.125	0.0058	0.038	0.09	0.104	0.077
140	106	0.106	0.0052	0.035	0.071	0.082	0.060
166	90	0.09	0.0046	0.032	0.063	0.072	0.054
200	75	0.075	0.0041	0.029	0.050	0.058	0.043
235	63	0.063	0.0037	0.026	0.045	0.052	0.038
282	53	0.053	0.0034	0.024	0.036	0.041	0.031
330	45	0.045	0.0031	0.022	0.032	0.037	0.027
391	38	0.038	0.0029	0.02	0.030	0.035	0.024

8) Sterilization and Aseptic Operation

(1) Sterilization and Aseptic Operation

Sterilization Sterilization refers to the killing or removal of all microorganisms from a substance. Sterilization methods generally involve consideration of the appropriateness of selecting the method, the procedure and conditions depending on the type of microorganism, the degree of contamination, and the nature and condition of the substance to be sterilized.
The appropriateness of sterilization is usually determined by the sterility test.
Sterilization procedures are performed after sufficient confirmation that the temperature, pressure, etc., are suitable for the desired sterilization conditions.
In addition, appropriate indicator organisms may be used for each sterilization method when selecting sterilization conditions or verifying the effectiveness of sterilization.

Aseptic operation Aseptic operation is used in the production of sterile drugs where terminal sterilization is not performed, which involves filling the drug into a final container (the container in which the drug will ultimately be used) and then sterilizing it. It is also used to produce sterile drugs after filtration sterilization or a series of sterilization procedures, starting with the raw material.
In the production of sterile drugs by this method, all instruments and materials to be used are usually sterilized in advance. Then, a certain sterile condition is maintained by using an appropriate aseptic procedure in a sterile facility so that the number of microorganisms and particles in the environment is properly controlled.

(2) Ultrafiltration

In ultrafiltration, water is filtered through a reverse osmosis membrane, an ultrafiltration membrane, or a combination of these membranes, which can remove all types of microorganisms and endotoxins.
The production of Water for Injection by ultrafiltration usually involves the use of pretreatment equipment, equipment for the production of water for injection, and equipment for the supply of water for injection. The pretreatment equipment is installed upstream of the equipment for the production of water for injection in order to reduce the load on the equipment for the production of water for injection by removing solids, dissolved salts, and colloids from the raw water. This equipment consists of an aggregation device, a sedimentation separation device, a filtration device, a chlorine sterilization device, an oxidation/reduction device, a residual chlorine removal device, a precision filtration device, a reverse osmosis device, an ultrafiltration device, and an ion exchange device. Depending on the quality of the raw water, there can be different combinations. The equipment for the production of water for injection consists of a device for the supply of pre-treated water, an ultraviolet sterilization device, a heat exchange device, a membrane filter, and a purification/sterilization device. The equipment for supplying water for injection consists of a water storage tank to respond to changes in use, piping for supplying Water for Injection to the point of use, a heat exchanger, a circulation pump, a pressure regulator, etc., and Water for Injection produced by ultrafiltration is sterilized by circulating and maintaining the temperature above 80 ℃ to prevent the growth of microorganisms.
When using this method to prepare Water for Injection, use a membrane filter that can remove microorganisms and substances with a molecular weight of about NLT 6000.