General Tests, Processes and Apparatus

General Tests, Processes and Apparatus

1. Alcohol Number Determination

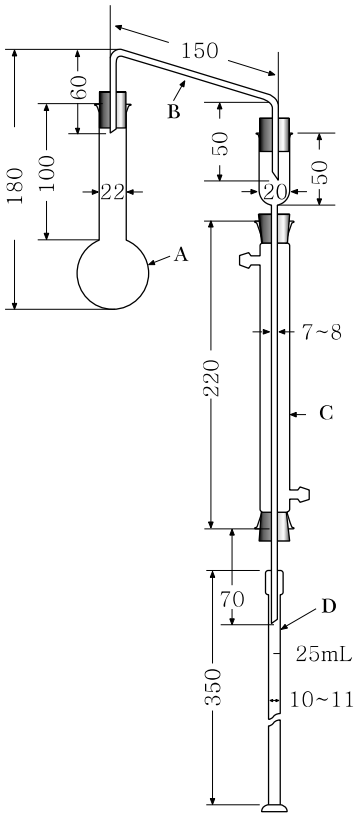
The Alcohol Number represents the number of milliliters of ethanol at 15 °C obtained from 10mL of tincture or other preparations containing ethanol by the following procedures.

Method 1. Distilling method

This is a method to determine the Alcohol Number by reading the number of milliliters of ethanol distillate at 15 °C obtained from 10mL of a sample measured at 15 °C by the following procedures.

(1) ***Apparatus:*** Use hard glass apparatus as illustrated herein. Ground glass may be used for the joints.

(2) ***Reagent:*** Alkaline phenolphthalein solution: To 1 g of phenolphthalein, add 7mL of sodium hydroxide TS and water to make 100mL.



The figures are in mm.

A: Distilling flask (50mL)
B: Delivery tube
C: Condenser
D: Glass-stoppered volumetric cylinder (25mL, graduated in 0.1mL)

Figure. Apparatus used or the Alcohol Number Determination.

(3) ***Procedure:*** Transfer 10mL of the sample preparation, accurately measured at 15 ± 2 °C, to the distilling flask, A, add 5mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder, D.

By reference to the following Table, a suitable volume of distillate (mL) should be collected, according to the content of ethanol in the sample preparation. Prevent bumping during distillation by rendering the sample strongly acidic with phosphoric acid or sulfuric acid, or by adding a small amount of paraffin, beeswax or silicone resin before starting the distillation. When the samples contain the following substances, carry out pretreatment as follows before distillation.

(i) Glycerin: Add sufficient water to the sample so that the residue in the distilling flask, after distillation, contains at least 50 % of water.

(ii) Iodine: Decolorize the sample with zinc powder.

(iii) Volatile substances: Preparations containing appreciable proportions of essential oil, chloroform, ether or camphor require treatment as follows. Mix 10mL of the sample, accurately measured, with 10mL of saturated sodium chloride solution in a separator, add 10mL of petroleum benzin, and shake. Collect the separated aqueous layer. The petroleum benzin layer was extracted with two 5mL volumes of saturated sodium chloride solution. Combine the aqueous layers, and distill. According to the ethanol content in the sample, collect a volume of distillate 2 to 3mL more than that shown in the below Table.

(iv) Other substances: Render preparations containing free ammonia slightly acidic with dilute sulfuric acid. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS, and if the preparations contain soap along with volatile substances, decompose the soap with an excess of dilute sulfuric acid before the extraction with petroleum benzin in the treatment described in (iii). To the distillate, add 4 to 6 g of potassium carbonate and 1 to 2 drops of alkaline phenolphthalein solution, and shake vigorously. If the aqueous layer shows no white turbidity, agitate the distillate with additional potassium carbonate. After allowing to stand in water at 15 ± 2 °C for 30 minutes, read the volume of the upper reddish ethanol layer inmL, and regard it as the Alcohol Number. If there is no clear boundary surface between these two layers, shake vigorously after addition of a few drops of water, then observe in the same manner.

Table. Amount of Distillate

Ethanol content in the sample (vol %)	Distillate to be collected (mL)
Above 80	13
80 - 70	12
70 - 60	11
60 - 50	10
50 - 40	9
40 - 30	8
Below 30	7

Method 2. Gas chromatography

This is a method to determine the Alcohol Number by determining ethanol (C_2H_5OH) content (vol %) from a sample measured at l5 °C by the following procedures.

(1) ***Reagent*** Dehydrated ethanol for Alcohol Number: Dehydrated ethanol with determined ethanol (C_2H_5OH) content. The relation between specific gravity, d_{15}^{15}, of dehydrated ethanol and ethanol (C_2H_5OH) content is 0.797: 99.46 vol %, 0.796: 99.66 vol %, and 0.795：99.86 vol %.

(2) ***Preparation of test solution and standard solution*** *Test solution* -Measure accurately a volume of sample at 15 ± 2 °C equivalent to about 5mL of ethanol (C_2H_5OH), and add water to make exactly 50mL. Measure accurately 25mL of this solution, add exactly 10mL of the internal standard solution, and add water to make 100mL.

Standard solution-Measure accurately 5mL of dehydrated ethanol for Alcohol Number at the same temperature as the sample, and add water to make exactly 50mL. Measure accurately 25mL of this solution, add exactly 10mL of the internal standard solution, and add water to make 100mL.

(3) ***Procedure*** Place 25mL each of the test solution and the standard solution in a 100-mL, narrowmouthed, cylindrical glass bottle sealed tightly with a rubber closure and aluminum band, immerse the bottle up to the neck in water, allowed to stand at room temperature for more than 1 hour in a room with little change in temperature, shake gently so as not to splash the solution on the closure, and allow to stand for 30 minutes. Perform the test with 1mL each of the gas in the bottle with a syringe according to the Gas Chromatography under the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of ethanol to that of the internal standard.

$$\text{Alcohol Number} = \frac{Q_T}{Q_S} \times \frac{5\,(\text{mL})}{\text{a volume}\,(\text{mL})\ \text{of sample}} \times \frac{\text{ethanol}\,(C_2H_5OH)\ \text{content (vol\%) of dehydrated ethanol for Alcohol Number}}{9.406}$$

Internal standard solution -A solution of acetonitrile (3 in 50).

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass tube, about 3 mm in inside diameter and about 1.5 m in length, packed with 150 μm to 180 μm porous ethylvinylbenzenedivinylbenzene copolymer for gas chromatography.

Column temperature: A constant temperature between 105 °C and 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: Proceed with 1mL of the gas obtained from the standard solution in the bottle under the above operating conditions, and calculate the resolution. Use a column giving elution of ethanol and the internal standard in this order with a resolution between their peaks being not less than 2.0.

2. Ammonium Limit Test

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

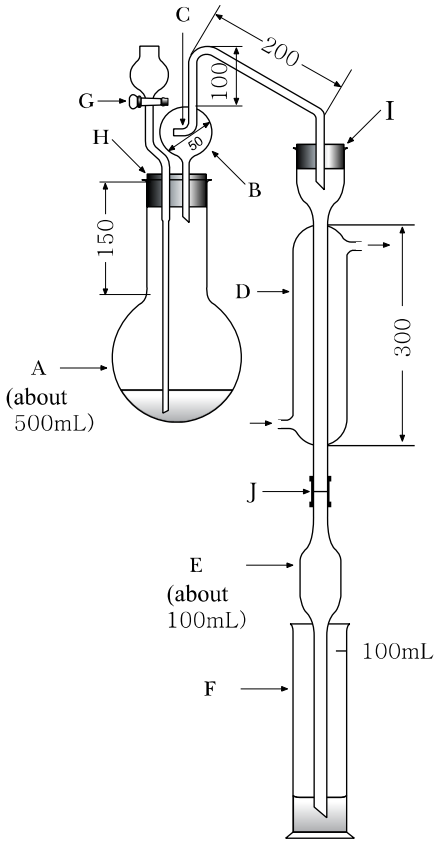
In the monograph, the permissible limit for ammonium (as NH_4^+) is described in terms of percentage (%) in parentheses.

Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Figure 1. For the distillation under reduced pressure, use the apparatus shown in Figure 2. Either apparatus is composed of hard glass, and ground-glass joints may be used. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and 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. Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver F, add 20mL of boric acid solution (1 in 200) as an absorbing solution, and immerse the lower end of the condenser. Adjust the heating to give a rate of 5 to 7mL per minute of distillate, and distill until the distillate measures 60mL. Remove the receiver from the lower end of the condenser, rinsing the end part with a small quantity of water, add sufficient water to make 100mL and designate it as the test solution.



The figures are in mm.

A: Distilling flask
B: Spray trap
C: Small hole
D: Condensor
E: Trap
F: Measuring cylinder
G: Stop cock
H,I: Rubber stoppers
J: Rubber tubing

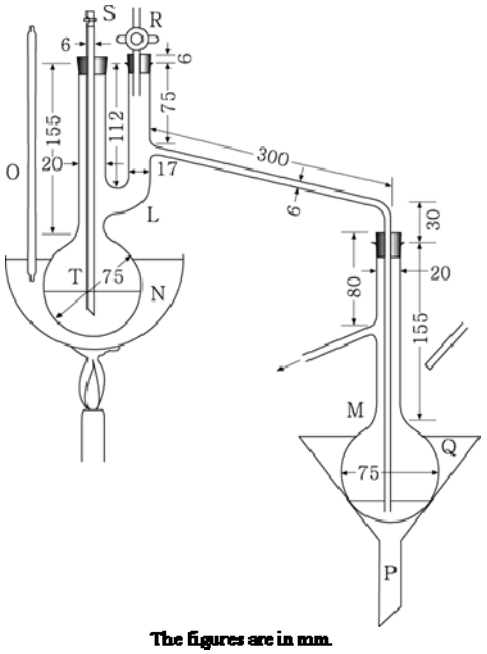
Figure 1. Distilling apparatus for the Ammonium Limit Test.

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

Place a volume of standard ammonium solution, directed in the monograph, in the distillation flask A or the vacuum distillation flask L, proceed as for the preparation of the test solution, and designate it as the control solution.

(2) ***Test of the test solution and the control solution*** Unless otherwise specified, proceed as directed in the following.

Place 30mL each of the test solution and the control solution in Nessler tubes, add 6.0mL of phenolsodium pentacyanonitrosylferrate (Ⅲ) TS to each solution, and mix. Then add 4mL of sodium hypochloritesodium hydroxide TS and water to make 50mL, mix, and allow to stand for 60 minutes. Compare the color of both solutions against a white background by viewing 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.

L: Vacuum distillation flask (200mL)
M: Receiver (a 200mL 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

Figure 2. Vacuum distilling apparatus for the Ammonium Limit Test.

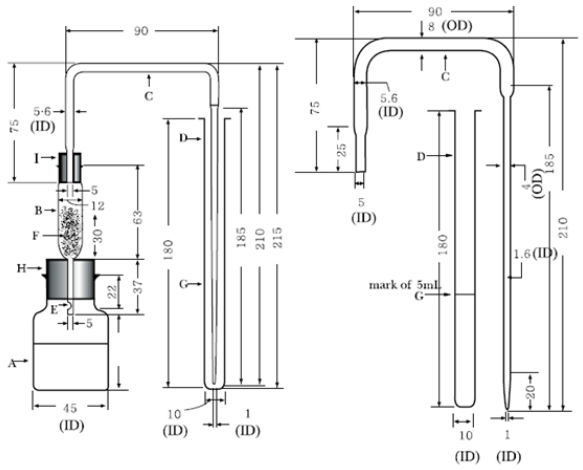
3. Arsenic Limit Test

The Arsenic Limit Test is a limit test for arsenic contained in drugs. The limit is expressed in terms of arsenic trioxide (As_2O_3). In each monograph, the permissible limit for arsenic (As_2O_3) is described in terms of ppm in parentheses.

Apparatus

Use the apparatus illustrated in Figure.

Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of lead acetate TS and water, and apply gentle suction to the lower end to remove the excess of the mixture. Insert the tube vertically into the center of the rubber stopper H and attach the tube to the generator bottle A so that the small perforation E in the lower end of B extends slightly below. At the upper end of B, attach the rubber stopper I to hold the tube C vertically. Make the lower end to the exit tube of C level with that of the rubber stopper I.



The figures are in mm.

A: Generator bottle (capacity up to the shoulder:approximately 70mL
B: Exit tube
C: Glass tube (inside diameter: 5.6mm, the tip of the part to be inserted in the absorber tube D is drawn out to 1 mm in diameter)
D: Absorber tube (inside diameter: 10 mm)
E: Small perforation
F: Glass wool (about 0.2 g)
G: Mark of 5mL
H,I: Rubber stoppers

Figure. Apparatus for the Arsenic Limit Test.

Preparation of the test solution

Unless otherwise specified, proceed as directed in the following.

(1) ***Method 1*** Weigh the amount of the sample directed in the monograph, add 5mL of water, dissolve by heating if necessary, and designate the solution as the test solution.

(2) ***Method 2*** Weigh the amount of the sample directed in the monograph, add 5mL of water, and add 1mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10mL of sulfurous acid, transfer to a small beaker, and evaporate the mixture on a water-bath until it is free from sulfurous acid and is reduced to about 2mL in volume. Dilute with water to make 5mL, and designate it as the test solution.

(3) ***Method 3*** Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10mL of a solution of magnesium nitrate in ethanol (1 → 50), ignite the ethanol and heat gradually to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate. After cooling, add 3mL of hydrochloric acid, heat on a water–bath to dissolve the residue, and designate it as the test solution.

(4) ***Method 4*** Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10mL of a solution of magnesium nitrate in ethanol (1 → 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3mL of hydrochloric acid, heat on a water–bath to dissolve the residue, and designate it as the test solution.

(5) ***Method 5*** Weigh the amount of the sample directed in the monograph, add 10mL of N,N-dimethylformamide, dissolve by heating if necessary, and designate the solution as the test solution.

Reagents

(1) ***Absorbing solution for hydrogen arsenide*** Dissolve 0.50 g of silver diethyldithiocarbamate in pyridine to make 100mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.

(2) ***Standard arsenic stock solution*** Weigh accurately 0.100 g of finely powdered arsenic trioxide dried at 105 °C for 4 hours, and add 5mL of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000mL.

(3) ***Standard arsenic solution*** Pipet 10mL of standard arsenic stock solution, add 10mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000mL. EachmL of the solution contains 1 μg of arsenic trioxide (As_2O_3). Prepare standard arsenic solution just before use and preserve in a glass-stoppered bottle.

Procedure

Unless otherwise specified, proceed as directed in the following procedure. Carry out the preparation of the standard color at the same time.

Place the test solution in the generator bottle A and, if necessary, wash down the solution in the bottle

with a small quantity of water. Add 1 drop of methyl orange TS, and after neutralizing with ammonia TS, ammonia solution (28) or dilute hydrochloric acid, add 5mL of diluted hydrochloric acid (1 in 2) and 5mL of potassium iodide TS, and allow to stand 2 to 3 minutes. Add 5mL of acidic tin (Ⅱ) chloride TS, and allow to stand for 10 minutes. Then add water to make 40mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle. Transfer 5mL of the absorbing solution for hydrogen arsenide to the absorber tube D, insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25 °C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5mL, if necessary, and observe the color of the absorbing solution: the color produced is not more intense than the standard color.

Preparation of standard color -Measure accurately 2mL of standard arsenic solution in the generator bottle A. Add 5mL of diluted hydrochloric acid (1 in 2) and 5mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5mL of acidic stannous chloride TS, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The color produced corresponds to 2 μg of arsenic trioxide (As_2O_3) and is used as the standard.

Note: Apparatus, reagents, and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

4. Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometry is a method to determine the amount or the concentration of an element in the sample specimen being examined, by utilizing the phenomenon that atoms being in the ground state absorb the light at specific wavelength, characteristics on elements, when light passes through the layer of atomic vapor of the element to be determined.

Apparatus

Usually, the apparatus consists of a light source, a sample atomizer, a spectroscope, a photometer, and a recording system. Some are equipped with a background compensation system. As a light source, usually a hollow cathode lamp specified for each element is used and sometimes a discharge lamp is also used. There are three types of sample atomizer: the flame type, the electrothermal type, and the cold vapor type. The first one is composed of a burner and a gas-flow regulator, the second one is composed of an electric furnace and a power source, and the third one is composed of a mercury generator and an absorption cell. The third one is further classified into two subtypes, which differ in the atomizing method for mercury containing-compounds: one utilizes chemical reduction-vaporization and the other utilizes a thermal reduction-vaporization method. For the selection of an appropriate analytical wavelength in a spectroscope, a grating for light diffraction or an interference filter can be used. A photometer is composed of detector and signal treatment system. A recording system is composed of a display and a recording device. A background compensation system is employed for the correction of atmospheric effects on the measuring system. Several principles can be utilized for background compensation, using continuous spectrum sources, the Zeeman splitted spectrum, the nonresonance spectrum, or self-inversion phenomena.

Another special option such as a hydride generator and a heating cell can also be used for the analysis of selenium. As a hydride generator, a batch method and/or a continuous flow method can be applied. While as a heating cell, there are two kinds of cell: one for heating by flame and the other for heating by electric furnace.

Procedure

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

(1) *Flame type* Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Next, a mixture of a combustible gas and a supporting gas is ignited and the gas flow and/or pressure should be adjusted to optimum conditions. The zero adjustment of the detecting system must be done through nebulizing the blank solvent into the flame. After setting up the measuring system, the test solution prepared by the specified procedure is introduced into the frame and the light absorption at the characteristic wavelength of the element to be determined is measured.

(2) *Electrothermal type* Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Further, set an electric furnace to the appropriate temperature, electric current, and heating program, as directed separately in the monograph. When a suitable amount of sample is injected into the heated furnace with an appropriate stream of inert gas, the sample is dried and ashed, simultaneously with atomization of the metallic compound included in the specimen. The atomic absorption specified is observed and the intensity of absorption is measured.

(3) *Cold vapor type* Fit the mercury lamp to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and a slit-width. In the chemical atomization-vaporization method, a mercury containing compound in the test solution, prepared by the specified procedure,

is chemically reduced to metallic mercury by adding a proper reducing reagent to the closed vessel and the generated mercury is vaporized and introduced into the absorption cell with a flow of inert gas. In the thermal atomization-vaporization method, the sample specimen on a quartz dish is heated electrically and the generated atomic mercury is vaporized and introduced into the absorption cell with a flow of inert gas. Thus, in both methods, the generated atomic mercury is carried into the absorption cell as cold vapor and the intensity of the characteristic atomic absorption of mercury is measured.

Determination

Usually, proceed by any of the following methods. In the determination, the possibility of interference for various reasons and the background effect must be considered and avoided, if possible.

(1) ***Calibration curve method*** Prepare standard solutions at more than 3 concentration levels, measure the specific absorption due to these standard solutions, and prepare the calibration curve of the atomic absorption against the concentration. Then measure the atomic absorption due to the sample specimen, in which the concentration of the element to be determined should be adjusted to be within the concentration range of the standard solutions, and determine the amount or the concentration of the element to be examined using the calibration curve.

(2) ***Standard addition method*** To equal volumes of more than 3 test solutions, prepared as directed in the monograph, add a measured quantity of the standard solutions to produce a series of solutions containing increasing amounts of the element to be examined, and further add a solvent to make up a constant volume. Measure the atomic absorption for the respective solutions, and plot the obtained values on a graph with the added amount or the concentration on the abscissa and the absorbance on the ordinate. Extrapolate the linear plot obtained by linking the data points, and determine the amount or the concentration of the element to be examined from the distance between the origin and the point where the plot intersects with the abscissa. This method is available only when the calibration curve obtained by Method 1 is confirmed to be linear and to pass through the origin.

(3) ***Internal standard method*** Prepare a series of standard solutions of the element to be determined, each containing a definite amount of the internal standard element directed in the monograph. For these standard solutions, measure the atomic absorption due to the standard element and the internal standard element separately at the respective wavelengths under the same operating conditions, and obtain the ratio of absorbance by the standard element to that by the internal standard element. Prepare a calibration curve for the element to be determined, with the amount or the concentration of the standard element on the abscissa and the above-mentioned ratio of the absorbance on the ordinate. Then prepare test solutions, adding the same amount of the internal standard element as contained in the standard solutions. Measure the ratio of the absorbance due to the element to be determined to that due to the internal standard element under the same conditions as employed for preparing the calibration curve, and determine the amount or the concentration of the element being examined by using the calibration curve.

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

5. Bacterial Endotoxins Test

The Bacterial Endotoxins Test is a test to detect or quantitate bacterial endotoxins of gram-negative bacterial origin using a lysate reagent prepared from blood corpuscle extracts of horseshoe crab *(Limulus polyphemus*, *Tachypleus tridentatus*). There are two types of technique for this test: the gel-clot techniques, which are based on the gel formation by the reaction of the lysate TS with endotoxins, and the photometric techniques, which are based on endotoxin-induced optical changes of the lysate TS. The latter include the turbidimetric techniques, which are based on the change in lysate TS turbidity during gel formation, and chromogenic techniques, which are based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Proceed by any of the these techniques for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot techniques, unless otherwise indicated.

The test is carried out in a manner that avoids endotoxin contamination.

Apparatus

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process. Commonly used minimum time and temperature settings are 30 minutes at 250 °C. If employing plastic apparatus, such as multi-well plates and tips for micropipettes, use only that which has been shown to be free of detectable endotoxin and which does not interfere with the test.

Preparation of Standard Endotoxin Stock Solution

Prepare standard endotoxin stock solution by dissolving endotoxin RS in water for bacterial endotoxins (BET). Endotoxin is expressed in endotoxin unit (EU). One EU is equal to one international unit (IU) of endotoxin.

Preparation of Standard Endotoxin Solution

After mixing standard endotoxin stock solution thoroughly, prepare appropriate serial dilutions of standard endotoxin solution, using water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

Preparation of Test solutions

Unless otherwise specified, prepare test solutions by dissolving or diluting drugs, using water for BET. Test solutions for containers for medicines should be prepared according to other specified procedures. If necessary, adjust the pH of the solution to be examined so that the pH of the mixture of the lysate TS and test solution falls within the specified pH range for the lysate reagent to be used. This usually applied to a test solution with a pH in the range of 6.0 to 8.0. TSs or solutions used for adjustment of pH may be prepared using water for BET, and then stored in containers free of detectable endotoxin. The TSs or solutions must be validated to be free of detectable endotoxin and interfering factors.

Determination of Maximum Valid Dilution

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a test solution at which the endotoxin limit can be determined. Determine the MVD from the following equation:

$$\text{MVD} = \text{endotoxin limit} \times \frac{\text{concentration of sample solution}}{\lambda}$$

Endotoxin limit: The endotoxin limit for injections, defined on the basis on of dose, equals *K/M*, where *K* is a minimum pyrogenic dose of endotoxin per kg of body mass (EU/kg), values for *K* are set as in the following table. The K values for the intravenous route are applicable to drugs to be administered by any route other than those shown in the table.

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

M is equal to the maximum dose of product per kg per hour. M is expressed inmL/kg for products to be administered by volume, in mg/kg or mEq/kg for products to be administered by mass, and in Unit/kg for products to be administration route. For products to be administered by mass or by units, the endotoxin limit should be decided based on the labeled amount of the principal drug. Sixty kg should be used as the average body mass of an adult when calculating the maximum adult dose per kg. The pediatric dose per kg body mass should be used when this is higher than the adult dose.

Concentration of test solution: mg/mL in the case of endotoxin limit specified by mass (EU/mg); mEq/mL in the case of endotoxin limit specified by equivalent (EU/mEq); units/mL in the case of endotoxin limit specified by biological unit (EU/ unit);mL/mL in the case of endotoxin limit specified by volume (EU/mL).

λ : the labeled reagent sensitivity in the gel-clot techniques (EU/mL) or the lowest point used (EU/mL) in the standard regression curve of the turbidimetric or chromogenic techniques.

Gel-clot Techniques

The gel-clot techniques detect or quantify endotoxins based on clotting of the lysate TS in the presence of endotoxin. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate reagent sensitivity and for interfering factors as described under Preparatory testing.

(1) *Preparatory testing*

(i) Test for confirmation of labeled lysate reagent sensitivity The labeled sensitivity of lysate reagent is defined as the lowest concentration of endotoxin that is needed to cause the lysate TS to clot under the conditions specified for the lysate reagent to be used.

The test for confirmation of the labeled lysate reagent sensitivity is to be carried out when each new lot of lysate reagent is used or when there is any change in the experimental conditions which may effect the outcome of the test. Perform the test by the following procedures.

Prepare standard solutions having four concentrations equivalent to 2λ, 1λ, 0.5λ and 0.25λ 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 with an equal volume of one of the standard solutions (usually, 0.10mL aliquots) in each test. When single test vials or ampules containing lyophilized lysate reagent are used, keep the tubes (or containers such as vials or ampules) containing the reaction mixture usually at 37 ± 1 °C for 60 ± 2 minutes, avoiding vibration. To test the integrity of the gel after incubation, invert each tube or container through approximately 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A 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 standard solutions of four concentrations one set, test four replicates of the set.

The test is valid when 0.25λ of the standard solution shows a negative result in each set of tests. If the test is not valid, repeat the test after verifying the test conditions.

The endpoint is the last positive test in the series of decreasing concentrations of endotoxin. Calculate the geometric mean endpoint concentration using the following formula:

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

$\sum e$: the sum of the log endpoint concentrations of the dilution series used
f: the number of replicates

If the geometric mean endpoint concentration is not less than 0.5λ and not more than 2.0λ, the labeled sensitivity is confirmed, and is used in tests performed with this lysate.

(ii) *Test for interfering factors*

This test is performed to check for the presence of enhancing or inhibiting factors for the reaction in test solutions.

Following Table 1, prepare solutions A, B, C and D using a test solution under test. Test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Considering the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described in (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

This test must be repeated when there is any change in the experimental conditions which may affect the outcome of the test.

The test is valid if solutions A and D show no reaction and the result for solution C confirms the labeled sensitivity.

If the geometric mean of endpoint concentration of solution B is not less than 0.5λ and not greater than 2.0λ, the test solution being examined does not contain interfering factors and complies with the test for interfering factors. Otherwise the test solution interferes with the test.

If the sample under test does not comply with the test at the dilution, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a grater dilution of the sample to be examined. Furthermore, interference of the test solution or diluted test solution may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment.

To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen 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. Sample solution only.

*2 Sample solutions added with standard endotoxin (for testing interfering factors).

*3 Standard endotoxin solutions for confirmation of the labeled lysate reagent sensitivity.

*4 Negative control. Water for BET only.

(2) *Limit test*

Specified in the individual monograph based on the formation of a firm gel in the presence of endotoxin at above labeled lysate reagent sensitivity, this method tests whether a test solution contains endotoxin not greater than the endotoxin limit.

(i) Procedure

Prepare the solutions A, B, C and D according to Table 2. Making these four solutions one set, test two replicates of the set.

In preparing solutions A and B, use the test solutions complying with (ii) Test for interfering factors of (1) Preparatory testing. In concerning the test conditions including the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

Table 2

Solution	Concentration of added endotoxin in each solution / Solution to which endotoxin is added	No. 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 Sample solution for the limit test. The solution may be diluted not to exceed the MVD.

*2 Positive control. Sample solutions at the same dilution as solution A, containing standard endotoxin at a concentration of 2λ.

*3 Positive control. Standard endotoxin solution containing standard endotoxin concentration of 2λ.

*4 Negative control. Water for BET only.

(ii) Interpretation

The test is valid when both replicates of solution B and C are positive and those of solution D are negative.

The sample meets the endotoxin test of the test when a negative result is found for both replicates of solution A and vice versa.

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 test of the test when a positive result is found for both replicates of the test solution at a dilution less than the MVD. If the test is positive for the sample at a dilution less than the MVD, the test may be performed at a dilution equal to the MVD.

(3) Assay

The test measures endotoxin concentrations of test solutions by titration to an endpoint of gel formation.

(i) Procedure

Prepare solutions A, B, C and D according to Table 3. Making these four solutions one set, test two replicates of the set. When preparing solutions one set A and B, use test solutions complying with (ii) Test for interfering factors of (1) Preparatory testing. In concerning the test conditions, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

Table 3

Solution	Concentration of added endotoxin in each solution / Solution to which endotoxin is added	Diluent	Dilution factor	Concentration of added endotoxin after dilution	Number of replicates
A *1	0 λ /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 λ /Water for BET	-	-	-	2

*1 Sample solution for the Quantitative test. The dilution range of the dilution series may be changed as appropriate, but not exceeding the MVD.

*2 Positive control. Sample solutions at the same dilution as solution A diluted at the lowest dilution factor, containing standard endotoxin at a concentration of 2λ.

*3 Standard endotoxin solution for confirmation or the labeled lysate sensitivity.

*4 Negative control. Water for BET only.

(ii) Calculation and interpretation

The test is valid when the following three conditions are met: (a) both replicates of the negative control solution D are negative, (b) both replicates of the positive product control solution B are positive and (c) the geometric mean endpoint concentration of solution C is in the range of 0.5 λ to 2.0 λ.

The endpoint is defined as the maximum dilution showing the last positive test in the dilution series of solution A, and the endotoxin concentration of test solution is calculated by multiplying the endpoint dilution factor by λ.

Calculate the geometric mean endotoxin concentration of the two replicates, using the formula given under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

If none of the dilutions of solution A is positive, report the endotoxin concentration of test solution as less than λ× the lowest dilution factor of solution A.

If all dilutions are positive, the endotoxin concentration of solution A is reported as equal to or greater than the greatest dilution factor of solution A multiplied by λ

Calculate the endotoxin concentration (in EU permL, in EU per mg or mEq or in EU per Unit) of the sample, based on the mean endotoxin concentration of test solution. The sample complies with the Bacterial Endotoxin Test if the endotoxin concentration of the sample in both replicates meets the requirement for the endotoxin limit (in EU permL, in EU per mg or mEq or in EU per Unit) specified in the individual monograph.

Photometric techniques

(1) ***Turbidimetric technique*** This technique is measuring the endotoxin concentrations of test solutions based on the measurement of turbidity change accompanying gel formation of the lysate TS. This technique is classified as either endpoint-turbidometric or kinetic-turbidometric.

The endpoint-turbidometric technique is based on the quantitative relationship between the concentration of endotoxins and the turbidity of the reaction mixture at a specified reaction time.

The kinetic-turbidometric technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined turbidity of the reaction mixture or the rate of turbidity development.

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

(2) ***Chromogenic technique*** This technique measures the endotoxin concentrations based on the measurement of chromophore released from a synthetic

chromogenic substrate by the reaction of endotoxins with the lysate TS. This technique is classified as either endpoint-chromogenic or kinetic-chromogenic.

The endpoint-colorimetric assay is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period.

The kinetic-chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined absorbance (or transmittance) of the reaction mixture or the rate of color development.
The test usually carried out at 37 ± 1 °C.

(3) ***Preparatory testing*** To assure the precision and validity of the turbidometric or chromogenic techniques, perform both Test for assurance of criteria for the standard curve and Test for interfering factors, as indicated below.

(i) Test for assurance of criteria for the standard curve

The test is to be carried out when each new lot of lysate reagent is used or when there is any change in the experimental conditions which may affect the outcome or the test. Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve within the range of endotoxin concentrations indicated by the instructions for the lysate reagent used. Perform the test using at least three 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 standard curve.

The absolute value of the correlation coefficient, |r|, is greater than or equal to 0.980 for the range of endotoxin concentrations set up, the criteria for the standard curve are valid and the curve complies with the test.

If the test is not valid, repeat the test after verifying the test conditions.

(ii) Test for interfering factors

Prepare solutions A, B, C, and D according to 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.).

The test for interfering factors must be repeated when any condition changes, which is likely to influence the result of the test.

Table 4

Solution	Concentration of added endotoxin in each solution	Solution to which endotoxin is added	No. of test tubes or wells
A *1	0	Test solution	Not less than 2
B *2	Middle concentration of the standard curve	Test solution	Not less than 2
C *3	At least 3 concentrations	Water for BET	Each not less than 2
D *4	0	Water for BET	Not less than 2

*1 Sample solution only (for assaying endotoxin concentration in the sample solution). The sample solution may be diluted not to exceed the MVD.

*2 Standard solutions at the same dilution as solution A, containing added standard endotoxin at a concentration equal to or near the middle of the standard curve.

*3 Standard endotoxin solution at the concentration used in, follow the procedure under (i) Test for assurance of criteria for the standard curve of (3) Preparatry testing(for the standard curve).

*4 Negative control. Water for BET only.

The test is valid when the following conditions are met.

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

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

Calculate the recovery of the endotoxin added to solution B from the concentration found in solution B after subtracting the endotoxin concentration found in solution A.

When the recovery of the endotoxin added to solution B is within 50 % to 200 %, the test solution under test is considered to be free of interfering factors and the solution complies with the test.

When the endotoxin recovery is out of the specified range, the test solution under test is considered to contain interfering factors. If the sample under test does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the test solution or diluted test solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

(4) Assay

(i) Procedure Prepare solutions, A, B, C, and D according to Table 4, and follow the procedure described in (ii) Test for interfering factors of (3) Preparatory testing.

(ii) 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.

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

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 %.

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

(iii) Interpretation

The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample calculated from the mean endotoxin concentration of solution A meets the requirement of the endotoxin limit (in EU permL, in EU per mg or mEq or in EU per Unit) specified in the individual monograph.

6. Bioautography

Bioautography is a method to identify or to assay an active component among substances separated by paper chromatography or thin-layer chromatography through microbial techniques.

If necessary, the content can be usually specified by % in the specifications of the individual monograph. Unless otherwise specified, perform the test as follows.

Developing solvents, standard solution, test solution, media, test organism, and preparation of suspensions of test organism or spore- Specify in a monograph.

Preparation of a culture box- The dimension of stainless steel box is about 25 mm (height), 200 ~ 300 mm (width), and 350 ~ 500 mm (length). Before use, sterilize with glass cover.

Stationary phase- 1) Filter paper Use a filter paper of 12 ~ 14 cm width and about 40 cm length.

2) Thin-layer plate Prepare as directed under Thin-layer Chromatography.

Prodecures- Prepare a plate by pouring 200 ~ 300mL of an agar medium into the culture box, spread 100 ~ 150mL of a test organism or spore, allow to stand for more than 1 hour at not exceeding 5 °C. and use this box for the test. Perform the test under aseptic conditions. Divide origin line of filter paper or thin-layer plate into 4 equal sections and mark them by vertical lines. Spot 5 µL each of highest concentration standard solution, middle concentration standard solution, standard solution, and test solution on the center of origin line in each section from 1 to 4 by using micropipette, and air-dry. If necessary, place this filter paper or thin-layer plate in the chamber saturated with vapor of a developing solvent mixtures for 30 ~ 60 minutes, then develop the filter paper or thin-layer plate in an appropriate developing apparatus by using ascending or descending mode. An operating temperature is 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 room temperature for drying. After drying, place the filter paper or thin-layer plate on the 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 ~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.

Distance of development, R_f value, test value, calculation, and interpretation- Perform according to a corresponding monograph.

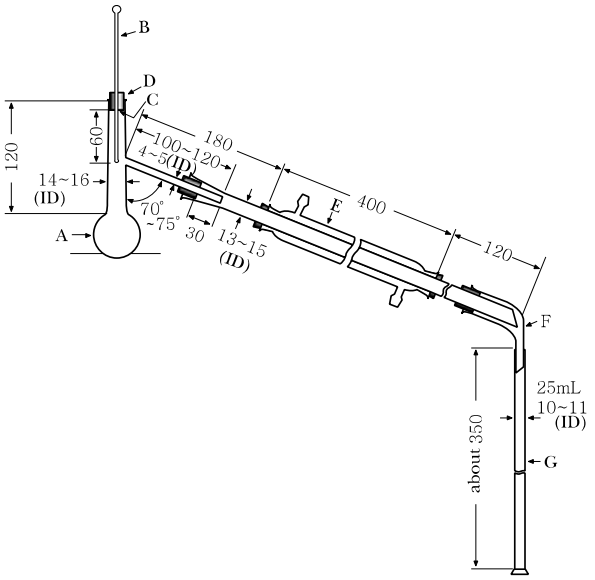
7. Boiling Point and Distilling Range Test

The Boiling Point and Distilling Range are determined by Method 1 or Method 2 as described herein, unless otherwise specified. Boiling point is the temperature shown between when the first 5 drops of distillate leave the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is done to determine the volume of the distillate which has been collected in the range of temperature directed in the monograph.

Method 1

This method is applied to a sample for which the permissible range of boiling temperature is smaller than 5 °C.

(1) ***Apparatus*** Use the apparatus illustrated in the Figure.



The figures are in mm.

A: Distilling flask
B: Thermometer with an immersion line
C: Immersion line D: Cork stopper
E: Condenser F: Adapter
G: Volumetric cylinder (25mL. graduated to 0.1mL)

Figure. Apparatus for the Boiling Point and Distilling Range Test

(2) *Procedure* Measure 25mL of the sample, whose temperature is previously noted, using a volumetric cylinder G graduated in 0.1mL, and transfer it to a distilling flask A of 50mL to 60mL capacity. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Put boiling chips into the distilling flask A, insert a thermometer B with an immersion line so that its immersion line C is on a level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube, and connect condenser E with the distilling flask A and adapter F with the condenser E. Insert the open end of F into the mouth of cylinder G (receiver) so that air can pass through slightly. Use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When direct flame is applied as the heat source, put A on a hole of a fire-resistant, heat-insulating board [a board consisting of a fire-resistant, heat-insulating material, 150 mm square and about 6 mm thick (or a wire gauge of 150 mm square bonded to fire-resistant, heat-insulating materials in about 6 mm thickness), having in its center a round hole 30 mm in diameter].

Unless otherwise specified, distil the liquid sample by the application of heat, at a rate of 4mL to 5mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than 200 °C and at a rate of 3mL to 4mL per minute in the case of liquids whose boiling temperature is 200 °C or over, and read the boiling point. For the distilling range test, bring the temperature of distillate to the temperature at which the volume was originally measured, and measure the volume of distillate.

Liquids that begin to distil below 80 °C are cooled to between 10 °C and 15 °C before measuring the volume, and the receiving cylinder is kept immersed in ice up to a point 25 mm from the top during the distillation.

Correct the observed temperature for any variation in the barometric pressure from the normal (101.3 kPa), by allowing 0.1 degree for each 0.36 kPa of variation, adding if the pressure is lower, or subtracting if higher than 101.3 kPa.

Method 2

This method is applied to the sample for which the permissible range of boiling temperature is 5 °C or more.

(1) *Apparatus* The same apparatus as described in Method 1 is used. However, use a 200-mL distilling flask A with a neck 18 mm to 24 mm in inside diameter having a delivery tube 5 mm to 6 mm in inside diameter. The fire-resistant, heat-insulating board used for direct flame heating should have in its center a round hole 50 mm in diameter.

(2) *Procedure* Measure 100mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 1mL, and carry out the distillation in the same manner as in Method l.

8. Chloride Limit Test

The Chloride Limit Test is a limit test for chloride contained in drugs.

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

Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, and dissolve it in a proper volume of water to make 40mL. Add 6mL of dilute nitric acid and water to make 50mL, and use this solution as the test solution. Separately, transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6mL of dilute nitric acid and water to make 50mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions by using the same procedure.

Add 1mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.

The opalescence developed in the test solution is not more than that of the control solution.

9. Conductivity Measurement

Conductivity Measurement is a method for the measuring the flowability of electric current in an aqueous solution. The measurement is made with a conductivity meter or a resistivity meter, and is used, for example, in the purity tests in monographs. The method is applied to evaluate the test item "Conductivity (Electrical Conductivity)" specified in the monographs. Further it is also used for monitoring the quality of water in the preparation of highly purified water. However, when applying this method for monitoring the quality of water, the details of measurement should be specified by the user, based on the principles described here.

Conductivity of a solution κ ($S \cdot m^{-1}$) is defined as the reciprocal of resistivity ρ (Ω·m), which is an indicator of the strength of ionic conductivity for a fluid conductor. Resistivity is defined as the product of electrical resistance per unit length and crosssection area of a conductor. When resistivity is ρ , cross-section area A (m^2), and length l (m), resistance R(Ω) can be expressed by the following equation,

$$R = \sigma \frac{l}{A}$$

Thus, conductivity κ is expressed as follows,

$$\kappa = \frac{1}{\rho} = \frac{1}{R} \times \frac{l}{A}$$

If l /A is known, the conductivity κ can be obtained by measuring resistance *R* or conductance G (= R^{-1}).

In the International System (SI), the unit of conductivity is the Siemens per meter ($S \cdot m^{-1}$). In practice, conductivity of a solution is generally expressed by $\mu S \cdot cm^{-1}$, and resistivity by Ω·m .

Unless otherwise specified, the reference temperature for the expression of conductivity or resistivity is 20 °C.

Items such as the sample preparation method, the necessity of blank correction, the calculation method, the specification value, and the measuring temperature should be described in the monograph, if necessary.

Apparatus

A conductivity meter or a resistivity meter is composed of an indicator part (operating panel, display, recording unit) and a detector part, the latter of which includes a conductivity cell. In the conductivity cell a pair of platinum electrodes is embedded. The cell is immersed in a solution, and the resistance or the resistivity of the liquid column between the electrodes is measured. Alternating current is supplied to this apparatus to avoid the effects of electrode polarization. Further, a temperature compensation system is generally contained in the apparatus.

Conductivity measurement is generally performed by using an immersion-type cell. A pair of platinum electrodes, the surfaces of which are coated with platinum black, is fixed in parallel. Both electrodes are generally protected by a glass tube to prevent physical shocks.

When the surface area of the electrode is *A* (cm^2), and the separation distance of the two electrodes is l (cm), the cell constant *C* (cm^{-1}) is given by the following equation.

$$C = \alpha \cdot \frac{l}{A}$$

α : a dimensionless numerical coefficient, and it is characteristic of the cell design.

In addition to the immersion-type cell, there are flow-through-type and insert-in-pipe-type cells. These cells are set or inserted in an appropriate position in the flow system for monitoring the quality of water continuously or intermittently, during the preparation of highly purified water.

Standard Solution of Potassium Chloride

After pulverizing an appropriate amount of potassium chloride for conductivity measurement, dry it at 500 ～ 600 °C for 4 hours. Take an indicated amount of the dried potassium chloride, as shown in Table 1, dissolve it in distilled or purified water (conductivity less than 2 $\mu S \cdot cm^{-1}$), previously boiled and cooled, and adjust to make 1000.0 g, for preparation of the standard solutions. The conductivity and the resistivity of the respective standard solutions at 20 °C are indicated in table 1. These standard solutions should be kept in tightly closed polyethylene or hard glass bottles.

Table 1. Conductivity and Resistivity of the Standard Solutions of Potassium Chloride at 20 °C

Concentration (g/1000.0 g)	Conductivity *κ* ($\mu S \cdot cm^{-1}$)	Resistivity ρ (Ω・cm)
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

When measurement at 20 °C can not be done, the standard solution (Table 1), can be corrected by using the equation below. However, the equation is valid only within the range of 15 ～ 30 °C.

$$K_T = K_{20}[1 + 0.021(T - 20)]$$

T : Measuring temperature specified in the monograph

k_T : Calculated conductivity of the KCl standard solution at °C($\mu S \cdot cm^{-1}$)

k_{20} : Conductivity of the KCl standard solution at 20 °C(μS·cm^{-1})

Operating Procedure

(1) Cell Constant

An appropriate conductivity cell should be chosen according to the expected conductivity of the sample solution. The higher the expected conductivity, the larger the cell constant required for the conductivity cell, so that the electrical resistance is within the measuring range of the apparatus being used. Conductivity cells with a cell constant of the order of 0.1 cm^{-1} , 1 cm^{-1} , or 10 cm^{-1} , are generally used. For determination or confirmation of the cell constant, an appropriate KCl standard solution should be chosen and prepared, taking account of the expected conductivity of the sample solution to be measured. Rinse the cell several times with distilled water. Next, after rinsing the cell 2 ~ 3 times with the standard solution used for the cell constant determination, immerse the cell in the standard solution contained in a measuring vessel. After confirming that the temperature of the standard solution is maintained at 20 ± 0.1 °C or at the temperature specified in the monograph, measure the resistance R_{KCl} or the conductance G_{KCl} of the standard solution, and calculate the cell constant C (cm^{-1}) by use of the following equation.

$$C = R_{KCl} \cdot k_{KCl} \text{ or } C = k_{KCl}/G_{KCl}$$

R_{KCl}: Measured resistance (MΩ)
G_{KCl}: Measured conductance (μS)
k_{KCl}: Conductivity of the standard solution being used (μS・cm^{-1})

The measured cell constant should be consistent with the given value within 5 %. If it is not consistent, coat the electrodes with platinum black again, or replace the cell with a new one.

(2) Suitability Test for the Apparatus

Using an appropriate KCl standard solution according to the expected conductivity of the sample solution, perform the suitability test for the apparatus. Rinse the conductivity cell several times with distilled water, and rinse again 2 ~ 3 times with the selected standard solution. Fill the standard solution in the measuring vessel.

After confirming that the temperature of the measuring system is maintained at 20 ± 0.1 °C, measure the conductivity of the standard solution. When this measuring procedure is repeated several times, the average conductivity should be consistent with an indicated value in Table 1 within 5 %. Further, relative standard deviation should be less than 2 %.

(3) Measurement

After confirmation of the suitability of the apparatus, perform the conductivity measurement for the sample solution. Unless otherwise specified, the preparation method for sample solution should be as specified in the respective monograph. Rinse the conductivity cell several times with distilled water, and rinse again 2 ~ 3 times with sample solution. Immerse the cell in the sample solution placed in a measuring vessel. If necessary, agitate gently the sample solution. After confirming that the temperature of the sample solution is maintained at 20 ± 0.1 °C or at the temperature specified in the monograph, measure the resistance R_T (MΩ) or conductance G_T (μS) of the sample solution, and calculate the conductivity by using the following equation.

$$k_T = CG_T \text{ or } k_T = C/G_T$$

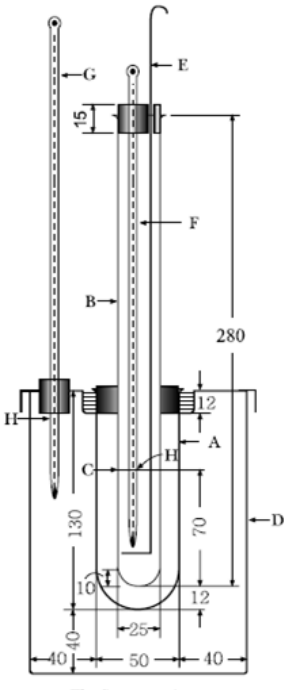
Items such as the sample preparation method, the necessity of blank correction, the calculation method, the specification value, and the measuring temperature should be described in the monograph, if necessary.

10. Congealing Point Determination

The Congealing Point is the temperature measured by the following method.

Apparatus

Use the apparatus illustrated in the figure.



The figures are in mm.

A: Cylinder made of glass (the tube is painted with silicone oil on both sides of the wall to prevent clouding).

B: Sample container (a hard glass test tube, which is painted with silicone oil to prevent clouding, except

at the region of the wall in contact with the sample; insert it into cylinder A, and fix with cork stopper).

C: A marked line.

D: Bath made of glass or plastics.

E: Stirring rod made of glass or stainless steel (3 mm in diameter, the lower end part of it is bent to make a loop, about 18 mm in diameter).

F: Thermometer with an immersion line.

G: Thermometer with an immersion line or a total immersion thermometer.

H: Immersion line.

Procedure

Transfer the sample into sample container B up to the marked line C. When the sample is solid, melt the sample by heating to a temperature not higher than 20 °C above the expected congealing point, and transfer to B. Fill the glass or plastic bath D with water at a temperature about 5 °C below the expected congealing point. When the sample is liquid at room temperature, fill bath D with water at a temperature between 10 °C and 15 °C lower than the expected congealing point.

Insert the sample container B containing the sample into cylinder A. Adjust the immersion line H of thermometer F to the same level of the meniscus of the sample. After cooling the sample to about 5 °C above the expected congealing point, move vertically the stirrer E at the rate of about 60 to 80 strokes per minute, and observe the thermometer readings at 30 second intervals. The temperature falls gradually. Discontinue stirring, when an appreciable amount of crystals has formed and the temperature is constant or has begun to rise. Usually, read the maximum temperature (reading of F), that is constant for a while after a rise of temperature. If no rise of temperature occurs, read the temperature that is constant for a while. The average of not less than four consecutive readings that lie within a range of 0.2 °C constitutes the congealing point.

Note: If a state of super cooling is anticipated, rub the inner wall of bath B or put a small fragment of the solid sample into bath B for promoting the congealment, when the temperature approaches near the expected congealing point.

11. Crystallinity Test

Crystallinity test is a method for the measurement of the crystallinity of the sample using a phasecontrast microscope.

Procedure: Float the sample on a mineral oil, and perform the test by using an appropriate phasecontrast microscope. To small amount of the sample, add 1~2 drops of fluid paraffin, shake and mix, and observe the sample under a microscope in the course of rotating 90 °. If the sample is fine powder, immersion oil method is employed. If the sample has crystallinity, birefringence and quenching phenomenon is observed.

12. Determination of Specific Gravity and Density

The density ρ (g/mL or g/cm^3) means the mass per unit volume, and the relative density means the ratio of the mass of a sample specimen to that of an equal volume of a standard substance. The relative density is also called the specific gravity.

The specific gravity, $d_t^{t'}$, means the ratio of the mass of the sample specimen at t′ °C to that of an equal volume of water (H_2O) at t °C. Unless otherwise specified, the measurement is to be performed by Method 1, Method 2, or Method 4. When the specified value is accompanied with the term “about” in the monograph, Method 3 is also available.

Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10mL to 100mL, having a ground-glass stopper fitted with a thermometer, and a side inlettube with a marked line and a ground-glass cap.

Weigh a pycnometer, previously cleaned and dried, to determine its mass, M. Remove the stopper and the cap. Fill the pycnometer with the test solution, keeping them at a slightly lower temperature by 1 °C to 3 °C than the specified temperature t' °C, and stopper them, taking care not to leave bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the portion of the test solution above the marked line through the side tube, cap the side tube, and wipe the outside surface thoroughly. Measure the mass M_1 of the pycnometer filled with the test solution. Perform the same procedure, using the same pycnometer containing water, and note the mass M_2 at the specified temperature t °C. The specific gravity $d_t^{t'}$ can be calculated by use of the following equation.

$$d_t^{t'} = \frac{M_1 - M}{M_2 - M}$$

TFurther, when measurements for a test solution and water are performed at the same temperature (t °C = t' °C), the density of the test solution at the temperature t' °C ($\rho_T^{t'}$) can be calculated from the measured specific gravity $d_t^{t'}$: and the density of water at the temperature t' °C ($\rho_{S1}^{t'}$) indicated in the attached Table by using the following equation.

$$\rho_T^{t'} = \rho_{S1}^{t'} \cdot d_t^{t'}$$

Method 2. Measurement using a Sprengel Ostwald Pycnometer

A Sprengel-Ostwald Pycnometer is a glass vessel with a capacity of usually 1mL to 10mL. As shown in the figure, both ends are thick-walled fine tubes (inside

diameter: 1 mm ~ 1.5 mm, outside diameter: 3 mm ~ 4 mm), one of which, tube A, has a line C marked on it. Determine the mass of a pycnometer, M, previously cleaned and dried, by hanging it on the arm of a chemical balance with a platinum or aluminum wire D. Immerse the fine tube B in the test solution, which is at a lower temperature by 3 °C to 5 °C than the specified temperature t' °C. Attach rubber tubing or a ground-glass tube to the end of A, and suck up the test solution until the meniscus is above the marked line C, taking care to prevent bubble formation. Immerse the pycnometer in a water bath kept at the specified temperature t' °C for about 15 minutes, and then, by attaching a piece of filter paper to the end of B, adjust the level of the test solution to the marked line C. Take the pycnometer out of the water bath, wipe thoroughly the outside surface and determine the mass M_1 . By use of the same pycnometer, perform the same procedure for the standard solution of water. Weigh the pycnometer containing water at the specified temperature t °C, and note the mass M_2 . Calculate the specific gravity $d_t^{t'}$, according to the equation described in Method l.

Further, when measurements of specific gravity for a test solution and water are performed at the same temperature (t' °C = t °C), the density of test solution at temperature t' °C can be calculated by using the equation described in Method l.

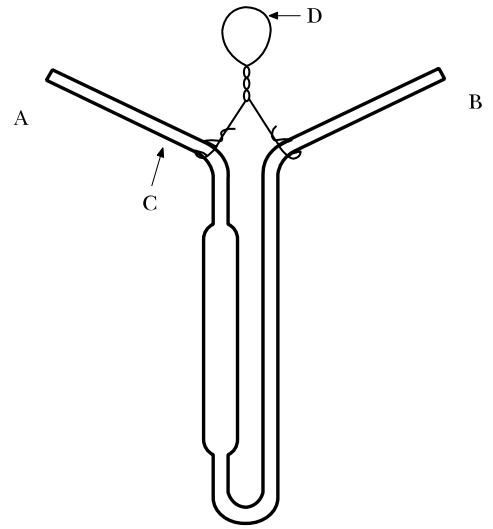


Figure 1. Sprengel-Ostwald pycnometer.

Method 3. Measurement using a hydrometer

Clean a hydrometer with ethanol or ether. Stir the sample well with a glass rod, and float the hydrometer in the well. When the temperature is adjusted to the specified temperature t' °C and the hydrometer comes to a standstill, read the specific gravity $d_t^{t'}$ or the density $\rho_T^{t'}$ at the upper brim of the meniscus. Here the temperature t °C indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a test solution is performed at the same temperature (t' °C = t °C), at which the hydrometer is calibrated, the density of a test solution at t' °C, $\rho_T^{t'}$, can be calculated by using the specific gravity $d_t^{t'}$ and the equation shown in Method l.

Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period T (s) of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period T in proportion to the mass of the sample specimen. If the volume of the vibrating part of the sample cell is fixed, the relation of the square of intrinsic oscillation period and density of the sample specimen shall be linear.

Before measuring a sample density, the respective intrinsic oscillation periods T_{S1} and T_{S2} for two reference substances (density: ρ_{S1} , ρ_{S2}) must be measured at a specified temperature t' °C, and the cell constant $K_{t'}$ (g ·cm^{-3}·s^{-2}) must be determined by using the following equation.

$$K_{t'} = \frac{\rho_{S1}^{t'} - \rho_{S2}^{t'}}{T_{S1}^{\ 2} - T_{S2}^{\ 2}}$$

Usually, water and dried air are chosen as reference substances. Here the density of water at t' °C, $\rho_{S1}^{t'}$, is taken from the attached Table, and that of dried air, $\rho_{S2}^{t'}$, is calculated by using the following equation, where the pressure of dried air, is at pkPa.

$$\rho_{S2}^{t'} = 0.0012932 \times \frac{273.15}{273.15 + t'} \times \frac{p}{101.325}$$

Next, introduce a sample specimen into a sample cell having a cell constant $K_{t'}$, the intrinsic vibration period, T_T , for the sample under the same operation conditions as employed for the reference substances. The density of a sample specimen at t' °C, $\rho_T^{t'}$, is calculated by use of the following equation, by introducing the intrinsic oscillation period T_{S1} and the density of water at a specified temperature t' °C, $\rho_{S1}^{t'}$, into the equation.

$$\rho_T^{t'} = \rho_{S1}^{t'} + K_t(T_T^{\ 2} - T_{S1}^{\ 2})$$

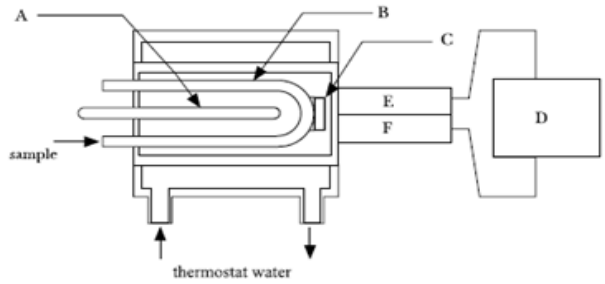
Further, the specific gravity of a sample specimen, $d_t^{t'}$, against water at a temperature t′ °C can be obtained by using the equation below, by introducing the

density of water at a temperature t °C, $\rho_{S1}^{t'}$, indicated 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 tube cell of about 1mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which applies an initial vibration to the cell, a detector for measuring the intrinsic vibration period, and a temperature controlling system.
A schematic illustration of the apparatus is depicted in Figure 2.



A: Thermometer
B: Sample cell
C: Vibration plate
D: Amplifier
E: Detector
F: Vibrator

Figure 2. An oscillator-type density meter

Procedure

A sample cell, water, and a sample specimen are previously adjusted 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, confirm that the temperature is at the specified value, and then measure the intrinsic oscillation period T_{S2} given by the dried air. Separately, the atmospheric pressure p (kPa) must be measured at the time and place of the examination. Next, introduce water into the sample cell and measure the intrinsic oscillation period T_{S1} given by water. Using these values of the intrinsic oscillation period and the atmospheric pressure, the sample cell constant $K_{t'}$ can be determined by use of the above-mentioned equation.

Next, introduce a sample specimen into the glass cell, confirm the specified temperature, and measure the intrinsic oscillation period T_T given by the sample specimen. Using the intrinsic oscillation periods for water and the sample specimen, the density of water $\rho_{S1}^{t'}$, and the cell constant $K_{t'}$, the density of the sample specimen, $\rho_T^{t'}$ can be obtained by use of the above equation. If necessary, the specific gravity of the sample specimen, $d_t^{t'}$ against water at a temperature t °C, can be calculated by using the density of water, $\rho_{S1}^{t'}$ shown in the attached Table.

In this measurement, avoid the occurrence of bubble formation in the sample cell, when a sample specimen or water is introduced into the cell.

Table. Density of water

Temp °C	Density g/mL	Temp °C	Density g/mL
0	0.99984		
1	0.99990	21	0.99799
2	0.99994	22	0.99777
3	0.99996	23	0.99754
4	0.99997	24	0.99730
5	0.99996	25	0.99704
6	0.99994	26	0.99678
7	0.99990	27	0.99651
8	0.99985	28	0.99623
9	0.99978	29	0.99594
10	0.99970	30	0.99565
11	0.99961	31	0.99534
12	0.99950	32	0.99503
13	0.99938	33	0.99470
14	0.99924	34	0.99437
15	0.99910	35	0.99403
16	0.99894	36	0.99368
17	0.99877	37	0.99333
18	0.99860	38	0.99297
19	0.99841	39	0.99259
20	0.99820	40	0.99222

* In this table, although the unit of density is represented by g/mL in order to harmonize with the unit expression in the text, it should be expressed in g/cm^3 seriously

13. Determination of Volume of Injection in Containers

Determination of Volume of Injection in Containers is performed to confirm that a slightly excess volume is filled for the nominal volume to be withdrawn. Injections may be supplied in single-dose containers such as ampoules or plastic bags, or in multi-dose containers filled with a volume of injection which is sufficient to permit administration of the nominal volume indicated on the label. The excess volume is determined by the characteristics of the product. Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the content. The contents are then cooled to between 20 °C and 25 °C before measuring the volume.

(1) Single-dose containers Select one container if the nominal volume is not less than 10mL, 3 containers

if the nominal volume is not less than 3mL and less than 10mL, or 5 containers if the nominal volume is less than 3mL. Take up individually the total contents of each container into a dry syringe of a capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardized dry cylinder graduated to contain, which is of such size that the volume to be measured occupies at least 40 % of its graduated volume. Alternatively, the volume of the contents in milliliters may be calculated as the mass in grams divided by the density. For containers with a nominal volume of not more than 2mL, the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement provided that a separate, dry syringe assembly is used for each container. The contents of containers holding not less than 10mL may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in case of containers examined individually, or in case of containers with a nominal volume of not more than 2mL, is not less than the sum of the nominal volumes of the containers taken collectively.

(2) Multi-dose containers For injections in multidose containers labeled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers using the same number of separate syringe assemblies as the number of doses specified.

The volume is such that each syringe delivers not less than the stated dose.

(3) Cartridges and prefilled syringes Select one container if the nominal volume is not less than 10mL, 3 containers if the nominal volume is not less than 3mL and less than 10mL, or 5 containers if the nominal volume is less than 3mL. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in milliliters calculated as the mass in grams divided by the density.

The volume measured for each of the containers is not less than the nominal volume.

(4) Parenteral infusions Select one container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40 % of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

14. Digestion Test

The Digestion Test is a test to measure the activity of digestive enzymes, as herbal materials or preparations, on starch, protein and fat.

Assay for Starch Digestive Activity

The assay for starch digestive activity is performed through the measurement of starch saccharifying activity, dextrinizing activity, and liquefying activity.

(i) *Measurement of starch saccharifying activity*

The starch saccharifying activity can be obtained by measuring an increase of reducing activity owing to the hydrolysis of the glucoside linkages when amylase acts on the starch. Under the conditions described in Procedure, one starch saccharifying activity unit is the amount of enzyme that catalyzes the increase of reducing activity equivalent to 1 mg of glucose per minute.

Preparation of Test Solution Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the reducing activity increases in proportion to the concentration of the test solution, when measuring under the conditions described in Procedure. The concentration is normally 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution Use potato starch TS for measuring the starch digestive activity. If necessary, add 10mL of buffer or salts solution specified in the monograph, instead of 10mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0).

Procedure Pipet 10mL of the substrate solution, stand at 37 ± 0.5 °C for 10 minutes, add exactly 1mL of the test solution, and shake immediately. Allow this solution to stand at 37 ± 0.5 °C for exactly 10 minutes, add exactly 2mL of alkaline tartrate solution of the Fehling' TS for amylolytic activity test, and shake immediately. Then, add exactly 2mL of copper solution of the Fehling' TS for amylolytic activity test, shake gently, heat the solution in a water bath for exactly 15 minutes, and then immediately cool to below 25 °C. Then, add exactly 2mL of concentrated potassium iodide TS and 2mL of diluted sulfuric acid (1 in 6), and titrate the released iodine with 0.05 mol/L sodium thiosulfate VS to the disappearance of the blue color produced by addition of 1 to 2 drops of soluble starch TS (amL). Separately, pipet 10mL of water instead of the substrate solution and titrate in the same manner (bmL).

$$\text{Starch saccharifying activity (unit/g)} = \text{amount(mg) of glucose} \times \frac{1}{10} \times \frac{1}{M}$$

Amount (mg) of glucose = (b −a) × 1.6

M: Amount (g) of sample in 1mL of the test solution

(ii) *Measurement of starch dextrinizing activity*

The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component (amylase) in starch when amylase acts on the starch. Under the conditions described in Procedure, one starch dextrinizing activity unit is the amount of enzyme required to reduce the coloration of potato starch by iodine by 10 % per minute.

Preparation of Test Solution Dissolve the sample in an appropriate amount of water or a buffer or salts solution specified in the monograph so that the coloration of starch by iodine decreases in proportion to the concentration of the test solution, when measuring under the conditions described in Procedure. The concentration is normally 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

Preparation of Substrate Solution Prepare the substrate solution in the same manner as the substrate solution in the measurement of starch saccharifying activity.

Procedure Pipet 10mL of the substrate solution, stand at 37 ± 0.5 °C for 10 minutes, add exactly 1mL of the test solution, and shake immediately. Allow this solution to stand at 37 ± 0.5 °C for exactly 10 minutes. Pipet 1mL of this solution, add it to 10mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5mL of this solution, add exactly 10mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance A_T of this solution at the wavelength of 660 nm as directed under Ultraviolet-visible Spectrophotometry. Separately, using 1mL of water instead of the test solution, determine the absorbance A_B in the same manner.

$$\text{Starch dextrinizing activity (unit/g) °C} = \frac{(A_B - A_T)}{A_B} \times \frac{1}{M}$$

M: Amount (g) of sample in 1mL of sample solution

(iii) ***Measurement of starch liquefying activity***

The starch liquefying activity can be obtained by measuring a decrease in the viscosity of starch solution resulting from the hydrolysis of molecules when amylase acts on the starch. Under the conditions described in Procedure, one starch liquefying activity unit is the amount of enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch from 200 % to 100 % of that of the 50 % sucrose standard solution.

Preparation of Test Solution Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the test solution, when measuring under the conditions described in Procedure. The concentration is normally 0.15 to 0.25 starch liquefying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution Weigh accurately about 1 g of potato starch, and measure the loss of drying at 105 °C for 2 hours. Weigh exactly potato starch equivalent to 15.00 g calculated on the dried basis, add 300mL of water, then add gradually 25mL of 2 mol/L sodium hydroxide TS under thorough shaking, until the mixture forms a paste. Heat the mixture in a water bath for 10 minutes, shaking occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

Preparation of 50 % Standard Sucrose Solution Dissolve 50.0 g of sucrose in 50.0mL of water.

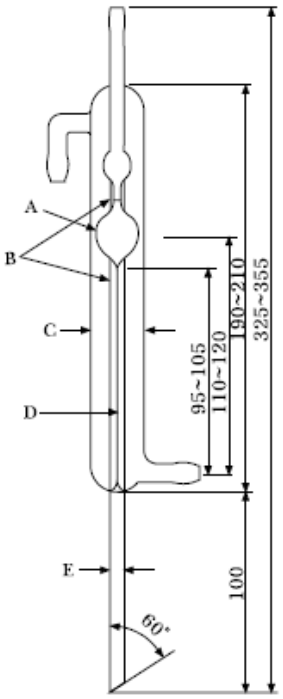
Procedure Put 50mL of the 50 % standard sucrose solution in a 100mL conical flask, and allow it to stand in a thermostat at 37 ± 0.5 °C for 15 minutes. Fix a viscometer shown in Figure 1 so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. After slowly drawing off the 50 % standard sucrose solution by suction to the middle of the upper bulb of the viscometer, let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t_1 seconds). Take exactly 50 g of the substrate solution in another 100mL conical flask, and stand it in another thermostat at 37 ± 0.5 °C for 20 minutes. Add exactly 1mL of the test solution to it, and shake the flask immediately. Fix a viscometer vertically so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. Occasionally draw the reaction solution off by suction to the middle of the upper bulb slowly, then let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t seconds).

Repeat this operation until t becomes shorter than t_1. At each measurement, record the time (T'seconds) from the moment that the test solution is added to the moment that the solution surface in the flask passes the upper indicator.

($T' + t/2$) is the reaction time (T) corresponding to t. Draw a curve for both t and T. Obtain T_1 and T_2 that corresponds to t_1 and ($2 \times t_1$) by interpolation.

$$\text{Starch liquefying activity (unit/g)} = \frac{60}{(T_1 - T_2)} \times \frac{1.5}{M}$$

M: Amount (g) of the sample in 1mL of the test solution



The figures are in mm.

A: Bulb volum: 5mL
B: Indicators
C: Outside diameter: 30 mm
D: Capillary inside diameter: 1.25 – 1.30 mm
E: Outside diameter: 8mm

Figure 1. Viscometer for the measurement of starch liquefying activity

(2) Assay for Protein Digestive Activity

The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin' reaction, of the amount of acid-soluble lowmolecular products, which is increased owing to the hydrolysis of the peptide linkages when protease acts on casein. One protein digestive activity unit is the amount of enzymes that produces Folin' TScolorable substance equivalent to 1 μg of tyrosine per minute under the conditions described in Procedure.

Preparation of Test Solution Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the amount of non-protein, Folin' TS-colorable substances increase in proportion to the concentration of the test solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 25 protein digestive activity unit/mL.

`Tyrosine Calibration Curve Weigh exactly 50 mg of tyrosine reference standard, previously dried at 105 °C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50mL. Pipet 1mL, 2mL, 3mL and 4mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make them exactly 100mL. Pipet 2mL of each solution, and add exactly 5mL of 0.55 mol/L sodium carbonate TS and 1mL of diluted Folin' TS (1 in 3) to each solution, shake immediately, then stand them at 37 ± 0.5 °C for 30 minutes. Determine the absorbances, A_1, A_2, A_3 and A_4, of these solutions at 660 nm as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared with exactly 2mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances, A_1, A_2, A_3 and A_4 as the ordinate, and with the amount (μg) of tyrosine for the absorbance 1.

Preparation of Substrate *Solution* Substrate solution 1: Weigh accurately abut 1 g of milk casein, and measure the loss on drying at 105 °C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 12mL of lactic acid TS and 150mL of water, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200mL. Prepare before use.

Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105 °C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160mL of 0.05 mol/L disodium hydrogenphosphate TS, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with the 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200mL. Prepare before use.

Preparation of Precipitation Reagent Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100mL.

Trichloroacetic acid TS B: Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5mL of 6 mol/L acetic acid TS in water to make 100mL.

Procedure Pipet 5mL of the substrate solution specified in the monograph, stand at 37 ± 0.5 °C for exactly 10 minutes, add exactly 1mL of the test solution, and shake immediately. After standing this solution at 37 ± 0.5 °C for exactly 10 minutes, add exactly 5mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at 37 ± 0.5 °C for 30 minutes, and then filter. Discard the first 3mL of the filtrate, exactly measure the subsequent 2mL of the filtrate, add exactly 5mL of 0.55 mol/L sodium carbonate TS and 1mL of diluted Folin' TS (1 in 3) to the solution, shake well, and stand it at 37 ± 0.5 °C for 30 minutes. Determine the absorbance A_T of this solution at 660 nm as directed under Ultravioletvisible Spectrophotometry, using water as the blank. Separately, pipet 1mL of the test solution, add exactly 5mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5mL of the substrate solution specified in the monograph, shake immediately, and stand it at 37 ± 0.5 °C for 30 minutes. Follow the same procedure for the test solution, and determine the absorbance A_B at 660 nm.

Protein digestive 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 the sample in 1mL of the test solution
F: Amount (μg) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

(3) Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1 μmole of fatty acid per minute under the conditions described in Procedure.

Preparation of Test Solution Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the test solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

Preparation of Substrate *Solution* Take 200 to 300mL of a mixture of emulsifier and olive oil (3:1) in a blender (Figure 2), and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below 10 °C. Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

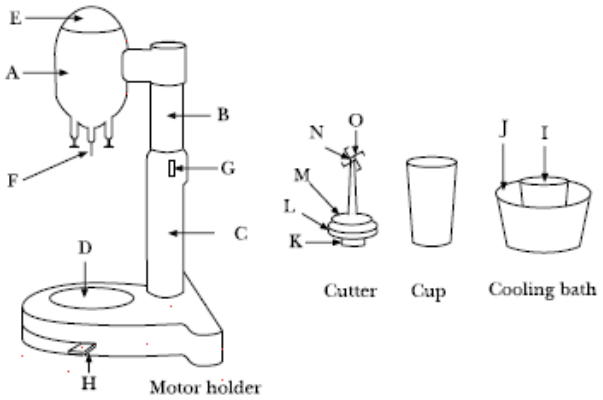
Preparation of Emulsifier Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800mL of water by heating between 75 °C and 80 °C for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000mL.

Procedure Pipet 5mL of the substrate solution and 4mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at 37 ± 0.5 °C for 10 minutes, add exactly 1mL of the test solution, and shake immediately. Stand this solution at 37 ± 0.5 °C for exactly 20 mintes, add 10mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (*b*mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5mL of the substrate solution and 4mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at 37 ± 0.5 °C for 10 minutes, add 10mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1mL of the testion solution and shake. Add exactly 10mL of 0.05 mol/L sodium hydroxide VS, and titrate in the same manner (*a*mL).

Fat digestive activity(unit/g)

$$=50\times(a-b)\times\frac{1}{20}\times\frac{1}{M}$$

M: Amount (g) of the sample in 1mL of the test solution



A: Motor case
B: Inside column
C: Outside column
D: Cooling bath mounting base
E: Motor top
F: Motor shaft
G: Motor ascend/descend lever
H: Rotation control lever
I: Cup mounting base
J: Cooling bath
K: Knob
L: Cup lid
M: Spout shield
N: Blade
O: Screw

Figure 2. Emulsifier for the assay for fat digestive activity

15. Disintegration Test

The Disintegration Test is provided to determine whether tablets, capsules, granules, pills or suppositories disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below. For the purposes of this test, disintegration does not imply complete solution of the unit or even of its active ingredient.

Apparatus

The apparatus consists of a basket-rack assembly, a 1000-mL, low-form beaker, 138 to 160 mm in height and having an inside diameter of 97 to 115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35 °C and 39 °C, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that 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 not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to that required

for the downward stroke. The change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-rack assembly: The basket-rack assembly consists of six open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 20.7 to 23.0 mm and a wall thickness of 1.0 to 2.8 mm; the tubes are held in a vertical position by two plastic plates, each 88 to 92 mm in diameter and 5 to 8.5 mm in thickness, with six holes, each 22 to 26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8- to 2.2-mm apertures and with a wire diameter of 0.57 to 0.66 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. The basket-rack assembly conforms to the dimensions illustrated in Figure 1. The design of the basket-rack assembly may be varied somewhat provided that the specifications for the glass tubes and the screen mesh sizes are maintained: for example, in order to secure the glass tubes and the upper and the lower plastic plates in position at the top or the bottom, an acid-resistant metal plate, 88 − 92 mm in diameter and 0.5 − 1 mm in thickness, having 6 perforations, each about 22 to 26 mm in diameter, may be used which coincide with those of the upper plastic plate and upper open ends of the glass tubes.

Auxiliary disk: The use of disks is permitted only where specified or allowed. Each tube is provided with a cylindrical disk 9.5 ± 0.15 mm thick and 20.7 ± 0.15 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five parallel 2 ± 0.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 6 ± 0.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped plans are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.6 ± 1.0 mm, and its bottom edges lie at a depth of 1.6 ± 0.1 mm from the cylinder' circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.4 ± 0.2 mm, and its center lies at a depth of 2.6 ± 0.1 mm from the cylinder' circumference. All surfaces of the disk are smooth. If the use of disks is specified, add a disk to each tube, and operate the apparatus as directed under Procedure. The disks conform to dimensions found in Figure 1. The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimension given in this chapter.

Auxiliary tube: The auxiliary tube, as illustrated in Figure 2, consists of a plastic tube D, 12 ± 0.2 mm inside diameter, 17 ± 0.2 mm in outside diameter, 20 ± 1 mm in length, having both outside ends screw-cut, and two plastic rings A, each 12 ± 0.2 mm in inside diameter, 17 ± 0.2 mm in outside diameter, 2.5 − 4.5 mm in length, having one inside end screw-cut. Acidresistant woven wire gauze having 0.42-mm openings and 0.29-mm wire diameter is placed in each plastic ring and the rings are attached by screws to each end of the plastic tube. The distance between two wire gauzes is 20 ± 1 mm. A handle of an acidresistant wire, 1 mm in diameter and 80 ± 5 mm in length, is attached to the mid proportion of the plastic tube. The auxiliary tube is used for the test of granules and capsules containing enteric coated granules.

Test fluids

1) First fluid Dissolve 2.0 g of sodium chloride in 7.0mL of hydrochloric acid, and add water to make 1000mL. This solution is clear and colorless, and its pH is about 1.2.

2) Second fluid To 250mL of 0.2 mol/L potassium dihydrogen phosphate TS, add 118mL of 0.2 mol/L sodium hydroxide TS and water to make 1000mL. This solution is clear and colorless, and its pH is about 6.8.

3) Water

Procedure

1) Immediate-release preparations

In case of tablets, capsules, and pills (except for pills containing herbal drugs), place 1 dosage unit in each of the six tubes of the basket, and if prescribed, add a disk. Unless otherwise specified, operate the apparatus, using water maintained at 37 ± 2 °C as the immersion fluid. In case the sample floats on the test fluid, a woven stainless steel wire cloth (0.57 to 0.66 mm in wire diameter), which has a plain square weave with 1.8- to 2.2-mm apertures, may be attached to the upper side of the upper plastic plate of the apparatus. Unless otherwise specified, carry out the test for 20 minutes for capsules, 30 minutes for plain tablets, and 60 minutes for coated tablets and pills. Lift the basket from the fluid, and observe the dosage units. Disintegration is achieved when any residue does not remain in the glass tube. Complete disintegration is also defined as that state in which any residue of the unit, except fragments from insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disks, if used, is a soft mass having no palpably firm core. The test is met if all of the dosage units have disintegrated completely. If 1 or 2 dosage units fail to disintegrate, repeat the test on 12 additional dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are disintegrated.

For pills containing herbal drugs, carry out the test for 60 minutes in the same manner, using the first fluid

as the immersion fluid. When any residue of the unit is observed, carry out the test successively for 60 minutes, using the second fluid.

In case of 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 tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using water maintained at 37 ± 2 °C as the immersion fluid, unless otherwise specified. Observe the samples after 30 minutes of operation for plain granules and after 60 minutes for coated granules, unless otherwise specified. Disintegration is achieved when any residue does not remain in the auxiliary tube. Complete disintegration is also defined as that state in which any residue of the granules, except fragments of insoluble coating in the auxiliary tube, is a soft mass having no palpably firm core. The test is met if all of 6 samples in the auxiliary tubes have disintegrated completely. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The test is met if not less than 16 of the total of 18 samples tested are disintegrated.

2) Enteric coated preparations

Unless otherwise specified, perform the following two tests, (a) the test with the first fluid and (b) the test with the second fluid, separately.

(1) Enteric coated tablet and capsule

(a) The test with the first fluid Carry out the test for 120 minutes, using the first fluid according to the procedure described in immediate-release preparations. Disintegration is defined as that state in which the tablet or capsule is broken or the enteric coating film is ruptured or broken. The test is met if none of 6 dosage units is disintegrated. If 1 or 2 dosage units are disintegrated, repeat the test on additional 12 dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are not disintegrated.

(b) The test with the second fluid According to the procedure described in immediaterelease preparations, carry out the test with new dosage units for 60 minutes, using the second fluid and determine if the test is met or not.

(2) Enteric coated granules and capsules containing enteric coated granules

Shake granules or contents taken out from capsules on 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 tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using the first and second fluids.

(a) The test with the first fluid According to the procedure described in immediaterelease preparations, carry out the test for 60 minutes, using the first fluid. The test is met if particles fallen from the openings of the wire gauze number not more than 15.

(b) The test with the second fluid According to the procedure described in immediaterelease preparations, carry out the test with new samples for 30 minutes, using the second fluid and determine if test is met or not.

3) Suppositories

Apparatus

As shown in Figure 3, the apparatus consists of a transparent sleeve of glass or suitable plastic, of height 60 mm with an internal diameter of 52 mm and wall thickness 8 mm, and a metal device consisting of two stainless metal discs, each of which contains 39 holes, each 4 mm in diameter. The diameter of the discs is closely similar to the internal diameter of the sleeve. The discs are separated by a distance of about 30 mm. The metal device is attached to the outer sleeve by means of three equally-spaced hooks.

Procedure

Place a suppository on the lower perforated disc of the metal device and then insert the device into the cylinder and attach this to the sleeve. Unless otherwise specified, place each piece of apparatus in three vessels each containing at least 4 L of water at about 36 °C or three pieces of apparatus in a vessel containing at least 12 L of water at about 36 °C and fitted with a slow stirrer and a means of holding the apparatus vertically 90 mm below the surface of the water. After each 10 minutes invert each apparatus without allowing it to merge from the liquid. Disintegration is considered to be achieved when the moulded suppository a) is completely dissolved or b) has separated into its component parts, which may collect on the surface (melted fatty substances), sink to the bottom (insoluble powders) or dissolve (soluble components) or may be distributed in one or more of these ways or c) has become soft, which may be accompanied by an appreciable change in shape, without necessarily separating completely into its components, and the mass has no solid core offering resistance to pressure with a glass rod.

Disintegration occurs in not more than 30 minutes for fat-based suppositories and in not more than 60 minutes for water-soluble suppositories, unless otherwise justified and authorized.

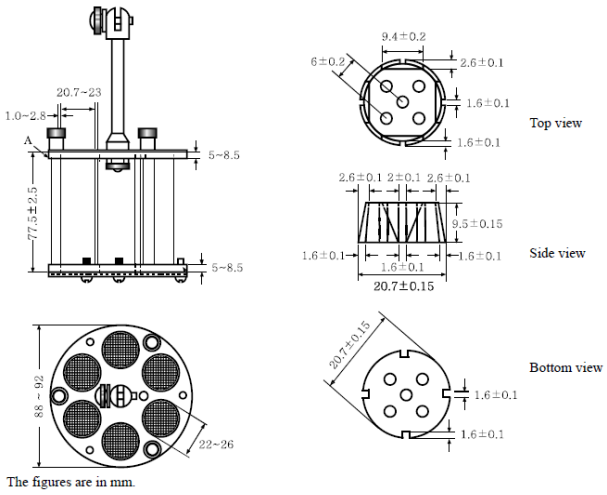


Figure 1. Disintegration apparatus

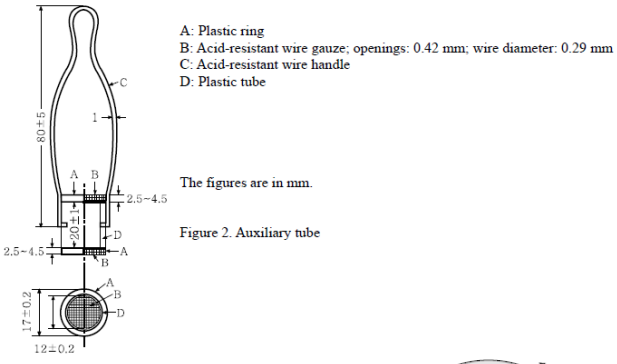


Figure 2. Auxiliary tube

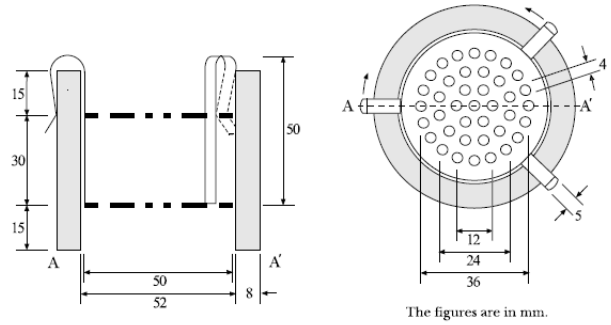


Figure 3. Apparatus for suppositories

16. Dissolution Test

The Dissolution Test is a method to test the dissolution of active ingredients from solid preparations for internal use, and aims at confirming the quality of solid preparations for internal use in relation to a fixed standard and also at preventing significant bioinequivalence. In this test, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified equivalent to minimum dose.

Apparatus

(1) *Method 1* *(Rotatory basket method, Apparatus 1)*

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material[*1] a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at 37 ± 0.5 °C during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element during the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to retard evaporation.[*2] The shaft is positioned so that its axes is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. Adjust a speed-regulating device to maintain the shaft rotation speed at a specified rate, within ± 4 %.

Shaft and basket components of the stirring element shown in figure 1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about 0.0001 inch (2.5 μm) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. 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 assembly from apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used if it provide that the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. When specified in the individual monograph, a small, loose piece of nonreactive material, such as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in figure 2a. Other validated sinker devices may be used. If the use of sinker is specified, unless otherwise specified, use the sinker device shown in figure 2a.

(3) *Method 3* *(Flow-through cell method, Apparatus 3)*

The assembly consists of a reservoir and a pump for the dissolution medium; a flow-through cell; a water bath that maintains the dissolution medium at 37 ± 0.5 °C. Use the cell size specified in the individual monograph.

The pump forces the dissolution medium upwards through the flow-through cell. The pump has a delivery range between 4 and 16mL per minute, with standard flow rates of 4, 8, and 16mL per minute. It must deliver a constant flow (± 5 percent of the nominal flow rate); the flow profile should be sinusoidal with a pulsation of 120 ± 10 pulses per minute. A pump without the pulsation can also be used.

The flow-through cell (see figures 3 and 4), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5 mm positioned at the apex to protect the fluid entry tube; a tablet holder (see Figures 3 and 4) is available for positioning of special dosage forms. The cell is immersed in a water bath, and the temperature is maintained at 37 ± 0.5 °C.

The apparatus uses a clamp mechanism of two Orings to assemble the cell. The pump is separated from the dissolution unit in order to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytef, with about 1.6 mm inner diameter and inert flanged-end connections.

(4) Apparatus suitability The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and temperature of the dissolution medium, rotation speed (Basket method and Paddle method), and flow rate of medium (Flow-through cell method).
Determine the acceptable performance of the dissolution test assembly periodically.

Dissolution medium

(1) *Solution 1* — dissolve 2.0 g of sodium chloride in 7.0mL of hydrochloric acid and water and fill water to 1000mL. This solution is colorless and transparent and pH of this solution is 1.2.

(2) *Solution 2* — mixture of phosphate buffer solution of pH 6.8 and water (1 : 1).

Procedure

(1) *Method 1 and Method 2*

(i) *Immediate-release dosage forms*

Procedure — Place the stated volume of the dissolution medium (± 1 %) in the vessel of the specified apparatus, assemble the apparatus, equilibrate the dissolution medium to 37 ± 0.5 °C, and remove the thermometer. Place 1 dosage unit in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operate the apparatus at the specific rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the Dissolution medium and the top of the rotating basket or blade, not less than 10 mm from the vessel wall. [NOTE –Where multiple sampling times are specified, replace the aliquots withdraw for analysis with equal volumes of fresh Dissolution medium at 37 °C or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.] Perform the analysis using an indicated assay method.[*3] Repeat the test with additional dosage units.

If automated equipment is used for sampling or the apparatus is otherwise modified, verification that the modified apparatus will produce results equivalent to those obtained with the standard apparatus described in this chapter, is necessary.

Dissolution Medium – A specified dissolution medium is used. If the dissolution medium is buffered solution, adjust the solution so that its pH is within 0.05 unit of the specified pH. [NOTE –Dissolved gases can cause bubbles to form, which may change the change of the results of the test. If dissolved gases influence the dissolution results, remove dissolved gases prior to testing.[*4]]

Time – Where a single time specification is given, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Specimens are to be withdrawn only at the stated times, within a tolerance of ± 2 %.

(ii) *Extended-release dosage forms*

Procedure—Proceed as described for Immediate-release dosage forms.

Dissolution medium—Proceed as directed under Immediate-release dosage forms.

Time—The test-time points, generally three, are expressed in hours.

(iii) *Delayed-release dosage forms*

Procedure — Unless otherwise specified, proceed the acid stage test and buffer stage test separately as described for immediate-release dosage forms.

Dissolution medium — Acid stage: Unless solution 1 for dissolution test is used, proceed as directed under immediate-release dosage forms. Buffer stage: Unless solution 2 for dissolution test is used, proceed as directed under Immediate-release dosage forms.

Time — Acid stage: Generally, test time is 2 hours for tablets and capsules, and 1 hour for granules. Buffer stage: The same as directed under Immediate- release dosage forms. All test times stated are to be observed within a tolerance of ± 2 %, unless otherwise specified.

(2) *Method 3*

(i) *Immediate-release dosage forms*

Procedure — Place the glass beads into the cell specified in the individual monograph. Place 1 dosage unit on top of the beads or, if specified, on a wire carrier. Assemble the filter head and fix the parts together by means of a suitable clamping device. Introduce by the pump the dissolution medium warmed to 37 ± 0.5 °C through the bottom of the cell to obtain the flow rate specified and measured with an accuracy of 5 %. Collect the eluent by fractions at each of the times stated. Perform the analysis as directed. Repeat the test with additional dosage units.

Dissolution medium — Proceed as directed in Immediate-release dosage forms under Method 1 and Method 2.

Time — Proceed as directed under Immediate-release dosage forms under Method 1 and Method 2.

(ii) *Extended-release dosage forms*

Procedure—Proceed as described for Immediate-release dosage forms.

Dissolution medium—Proceed as directed under Immediate-release dosage forms.

Time—The test-time points, generally three, are expressed in hours.

Interpretation

(1) *Immediate-release dosage forms*

Follow interpretation 1 when the value Q is specified in the individual monograph, otherwise follow interpretation 2.

(i) *Interpretation 1:* Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 1. Continue testing through the three stages unless the results conform at either S1 or S2.

The quantity, *Q*, is the specified amount of dissolved active ingredient, expressed as a percentage of the labeled content of the dosage unit; the 5 %, 15 %, and 25 % values in the Acceptance Table are percentage of the labeled content so that three values and *Q* are in the same terms.

Acceptance Table 1.

Stage	Number tested	Acceptance criteria
S1	6	Each value is not less than Q +5 %
S2	6	Average value of the 12 dosage units (S1 + S2) is equal to or greater than Q, and no value is less than Q –15 %.
S3	12	Average value of the 24 dosage units (S1 + S2 + S3) is equal to or greater than Q, not more than 2 values are less than Q –15 %, and no value is less than Q –25 %.

(ii) *Interpretation 2:* Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on 6 additional dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test.

Acceptance Table 2.

Level	Number tested	Criteria
L1	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time
L2	6	The average value of the 12 dosage units (L1 + L2) lies within each of the stated ranges and is not less than the stated amount at the final test time; no value is more than 10 % of labeled content outside each of the stated ranges; and no value is more than 10 % of labeled content below the stated amount at the final test time.
L3	12	The average value of the 24 dosage units (L1 + L2 + L3) lies within each of the stated ranges, and is not less than the stated amount at the final test time; not more than 2 of the 24 values are more than 10 % of labeled content outside each of the stated ranges; not more than 2 of the 24 values are more than 10 % of labeled content below the stated amount at the final test time; and no value is more than 20 % of labeled content outside each of the stated ranges or more than 20 % of labeled content below the stated amount at the final test time.

(2) *Extended-release dosage forms*

(i) *Interpretation 1:* Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 2. Continue testing through L3 unless the results conform at either L1 or L2. Limits on the amounts of active ingredient dissolved, are expressed as a percentage of the labeled content. The limits embrace each value of Q_i. The amount dissolved

at each specified fractional dosing interval. Where more than one range is specified, the acceptance criteria apply individually to each range.

(ii) *Interpretation 2:* Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on 6 additional dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. Where more than one range is specified, the acceptance criteria apply individually to each range.

(1) *Delayed-release dosage forms*

Follow interpretation 1 when the value Q is specified using solution 2 for dissolution test in the individual monograph, otherwise follow interpretation 2.

Acceptance Table 3.

Level	Number tested	Criteria
A1	6	No individual value exceeds 10 % dissolved.
A2	6	The average value of the 12 dosage units (A1 + A2) is not more than 10 % dissolved, and no value is greater than 25 % dissolved.
A3	12	The average value of the 24 dosage units (A1 + A2 + A3) is not more than 10 % dissolved, and no value is greater than 25 % dissolved.

(i) *Interpretation 1*

Test using solution 1 for dissolution test: Unless otherwise specified, the requirements of this portion of the test are met if the quantities, based on the percentage of the labeled content, of active ingredient dissolved from the units tested conform to Acceptance Table 3. Continue testing through A3 unless the result conforms at A2 that no value is greater than 25 % dissolved and the average value meet the requirement.

Test using solution 2 for dissolution test: Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 4. Continue testing through B3 unless the results conform at an earlier B1 or B2. The value of Q in Acceptance Table 4 is the amount specified in monograph of active ingredient dissolved, expressed as a percentage of the labeled content; the 5 %, 15 %, and 25 % values in the Acceptance Table 4 are percentages of the labeled contents so that these values and *Q* are in the same terms.

Acceptance Table 4.

Level	Number tested	Criteria
B1	6	No value is less than *Q* + *5 %*
B2	6	The average value of the 12 dosage units (B1 + B2) is equal to or greater than Q, and no value is less than Q – 15 %.
B3	12	The average value of the 24 dosage units (B1 + B2 + B3) is equal to or greater than Q, not more than 2 values are less than Q – 15 %, and no value is less than Q –25 %.

(ii) *Interpretation 2:* Unless otherwise specified, both the tests using solution 1 for dissolution test and solution 2 for dissolution test in acid and buffer stages, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on 6 additional dosage forms: if individual dissolution rates of not less than 10 samples out of 12 meet the requirements, the dosage forms conform to the test.

[*1] The materials should not sorb, react, or interfere with the specimen being tested.

[*2] If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

[*3] Test specimens are filtered immediately upon sampling unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the ingredient or contain extractable substances that would interfere with the analysis.

[*4] One method of deaeration is as follows: Heat the medium, while stirring gently, to about 41 °C, immediately filter under vacuum using a filter having a porosity of 0.45 μm or less, with vigorous stirring, and continue stirring under vacuum for about 5 minutes. Other validated deaeration techniques for removal of dissolved gases may be used.

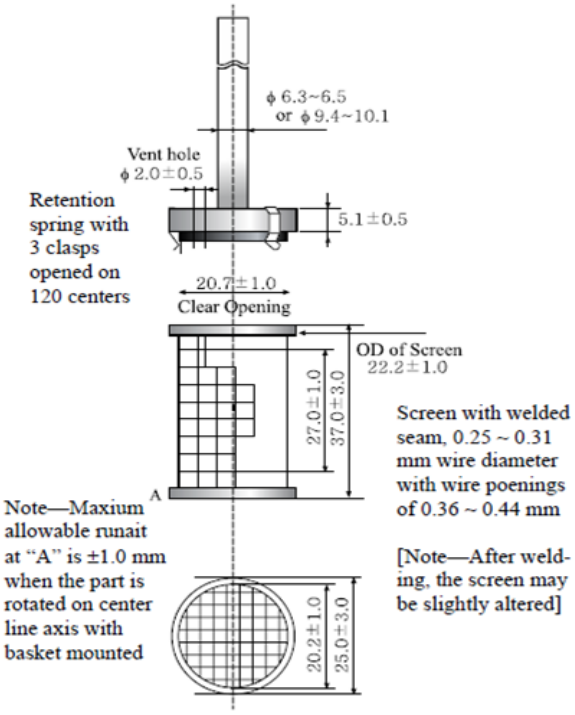
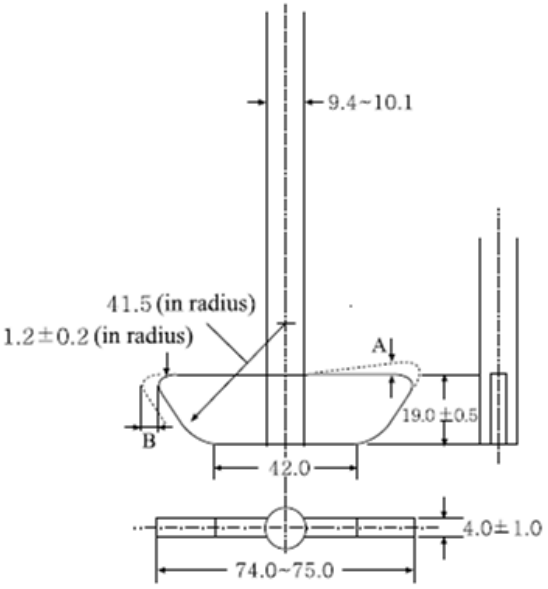


Figure 1. Apparatus 1, Basket stirring element

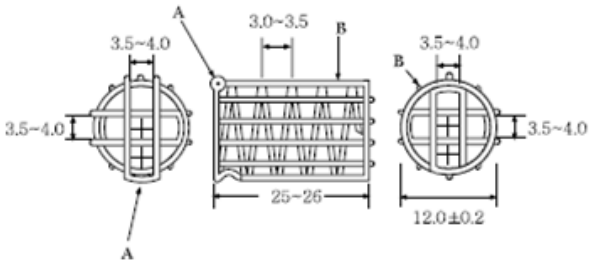


Notes :

(1) A and B dimensions are not to vary more than 0.5 mm when part is rotated on centering axis.

(2) Tolerances are ±1.0 mm unless otherwise stated.

Figure 2. Apparatus 2. Paddle stirring element

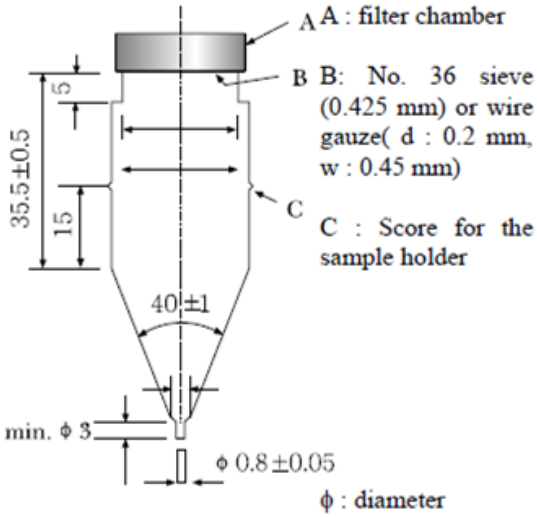


All dimensions are expressed in mm.

A: Acid-resistant wire clasp

B: Acid-resistant wire support

Figure 2a



The figures are in mm.

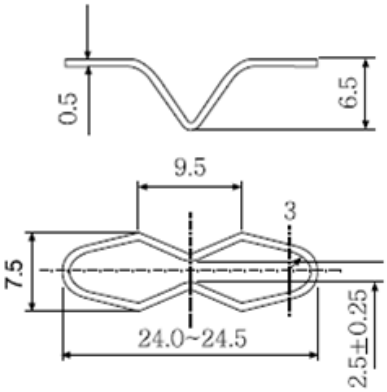


Figure 3. Apparatus 3

Large cell for tablets and capsules (top); tablet holder for the large cell (bottom)

(All dimensions are expressed in mm unless otherwise noted.)

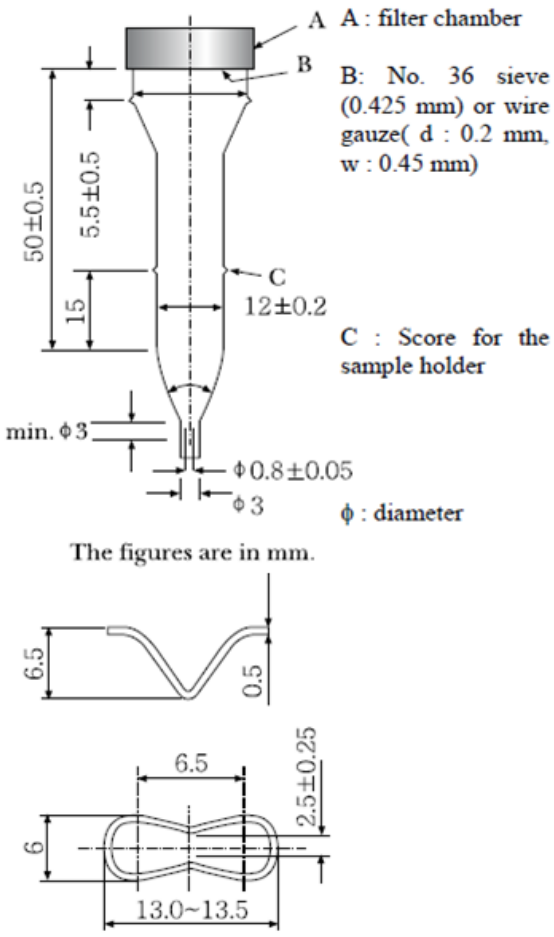


Figure 4. Apparatus 3.

Small cell for tablets and capsules (top); tablet holder for the small cell (bottom)
(All dimensions are expressed in mm unless otherwise noted.)

17. End point detection methods in Titrimetry

Titrimetry is a method or a procedure for volumetric analysis, which is usually classified into acid-base titration (neutralization titration or pH titration), precipitation titration, complexation titration, oxidation-reduction titration, etc., according to the kind of reaction or the nature of the phenomenon occurring between the titrate and the titrant (standard solution for volumetric analysis). Furthermore, titration performed in non-aqueous solvent is generally called non-aqueous titration, which is frequently used for volumetric analysis if weak acids, weak bases, and their salts. The end point in titrimetry can be detected by color changes of indicators and/or by changes of electrical signals such as electrical potential or electrical current.

The indicator method is one of the end point detection methods in titrimetry. In this method the color of an indicator dye, dissolved in the titrate, changes dramatically in the vicinity of the equivalence point due to its physico-chemical character, and this property is used for visual end point detection. Selection of an indicator and specification of the color change induced in the respective titration system, should be described in the individual monograph. An appropriate indicator should change color clearly, in response to a slight change in physico-chemical properties of the titrate, such as pH, etc., in the vicinity of the equivalence point.

Regarding the electrical end point detection methods, there are an electrical potential methods and an electrical current methods, which are called potentiometric and amperometric titration methods, respectively. They are generically named electrometric titration. In the potentiometric titration methods, the end point of a titration is usually determined to be the point at which the differential potential change becomes maximum or minimum as a function of the drop-wise quantity of titrant added. In the amperometric titration method, unless otherwise specified, a bi-amperometric reaction, as described in "Water Determination 2. Coulometric Titration".

The composition of a titration system, such as amount of specimen, solvent, standard solution for volumetric analysis, end point detection method, equivalent amount of titration method is used, and the end point is determined by following the change of microcurrent during the course of a titration. Furthermore, the quantity of electricity (electrical current × time) is often used as another electrochemical signal to follow a chemical substance to be examined (mg)/standard solution (mL), should be specified in the individual monograph. Standardization of the standard solution and titration of a specimen are recommended to be done at the same temperature. When there is a marked difference in the temperatures at which the former and the latter are performed, it is necessary to make an appropriate correction for the volume change of the standard solution due to the temperature difference.

Method I. Indicator method

Weigh an amount of a specimen in a flask or a suitable vessel as directed in the monograph or in "Standard Solutions for Volumetric analysis", and add a specified quantity of solvent to prepare the titrant, titrate by adding a standard solution for volumetric analysis by using a buret. In the vicinity of the end point, observe the color induced by the cautious addition of 0.1mL or less of the titrant. Calculate the drop-wise quantity of titration added from the readings on the scale of the buret used for the titration at the starting point and at the end point at which the specified color change appears, as directed in the individual monograph or in the "Standard Solutions for Volumetric analysis". Although addition of the volumetric standard solution by buret is usually done manually, an automatic buret can also be used.

Unless otherwise specified, perform a blank determination according to the following method, and make any necessary correction.
Measure a specified quantity of solvent, as directed in the monograph or in the "Standard Solutions for Volumetric analysis", and titrate as directed. The required drop-wise quantity of the standard solution added to reach a specified color change, is assumed to be the blank quantity for the titration system. However, when the blank quantity is too small to evaluate accurately, the quantity can be assumed to be zero.

Method II. Electrical End point Detection Methods

1. Potentiometric titration

(1) *Apparatus*

The Apparatus consists of a beaker to contain the specimen, the drop-wise buret for adding standard solution, an indicator electrode and a reference electrode, a potentiometer for measuring potential difference between the electrodes or an adequate pH meter, a recorder, and a stirrer for gently stirring of the solution to be examined. separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

In this titration method, unless otherwise specified, indicator electrodes designated in Table 1 are used according to the kind of titration. As a reference electrode usually a silver-silver chloride electrode is used. Beside the single indicator electrodes as seen in Table 1, a combined reference electrode and indicator electrode can also be used.

Table 1. Kinds of Titration and Indicator Electrodes

Kind of titration	Indicator electrode
Acid-base titration (Neutralization titration, pH titration)	Glass electrode
Precipitation titration (Titration of halogenion by silver nitrate)	Silver electrode. A silver-silver chloride electrode is used as a reference electrode, which is connected with the titrate by a salt bridge of saturated potassium nitrate solution.
Oxidation-reduction titration (Diazo titration etc.)	Platinum electrode
Complexation titration (Chelometric titration)	Mercury-mercury chloride (II) electrode
Non-aqueous titration (Perchloric acid titration, tetramethylammonium titration)	Glass electrode

When the potentiometric titration is carried out by the pH measurement method, the pH meter should be adjusted according to the "pH Determination".

(2) Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the monograph. After the potential difference ***E*** (mV) or the pH value of the solvent to be used for titration has reached a stable value, immerse both reference and indicator electrodes, which have previously been washed with the solvent being used, in the solution to be examined, and titrate with a standard solution for volumetric analysis with gentle stirring of the solution. During the titration, the tip of a buret should be dipped into the solution, to be examined. The end point of titration is determined by following the variation of the potential difference between two electrodes as a function of the drop-wise quantity of a titrant added. In the vicinity of the end point, the drop-wise amount of a titrant added should be 0.1mL or less for adequate titrimetry. Plot the obtained potential values along the ordinate and the drop-wise quantity of a titrant added ***V*** (mL) along the abscissa to draw a titration curve, and obtain the end point of titration from the maximum or the minimum value of *DE/DV* or from the value of electromotive force or pH corresponding to the equivalence point.
Unless otherwise specified, the decision of the end point in this method is usually made by either of the following methods.

(i) *Drawing method* Usually, draw two parallel tangent lines with a slope of about 45º to the obtained titration curve. Next, draw a 3rd parallel line at the same distance from the previously drawn two parallel lines, and decide the intersection point of this line with titration curve. Further, from the intersection point, draw a vertical line the abscissa, and read the drop-wise quantity of titrant added as the end point of the titration.
Separately, the end point of the titration can also be obtained from the maximum or the minimum of the differential titration curve ($\Delta E/\Delta V$ vs. *V)*.

(ii) *Automatic detection method* In the case of potentiometric titration using an automatic titration system, the end point can be determined by following the respective instrumental indications. The end point is decided either by following the variation of the differential potential change or the absolute potential difference as a function of the drop-wise quantity of titrant added: in the former case the drop-wise quantity given by the maximum or the minimum of the differential values, and in the latter the drop-wise quantity given by the indicator reaching the end point potential previously set for the individual titration system, are assumed to be the end point volumes, respectively.

2. Amperometric titration

(1) *Apparatus* The apparatus consists of a beaker for holding a specimen, a drop-wise buret for adding a standard solution for volumetric analysis, two small platinum plates or wires of the same shape as the indicator electrode, a device to load direct current microvoltage between two electrodes, a microammeter to measure the indicator current between two elec-

trodes, a recorder, and a stirrer which can gently stir the solution in a beaker. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

(2) *Procedure*

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the individual monograph. Next, after washing the two indicator electrodes with water, immerse both electrodes in the solution to be examined, apply a constant voltage suitable for measurement across two electrodes by using an appropriate device, and titrate the solution with a standard solution for volumetric analysis. During the titration, the tip of a drop-wise buret should be dipped into the solution to be examined. The end point of titration is determined by following the change of microcurrent between the two electrodes as a function of the drop-wise quantity titrant added. In the vicinity of the end point, the drop-wise amount of the titrant added should be 0.1mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the drop-wise quantity of the titrant added ***V*** (mL) along the abscissa to draw a titration curve, and usually take the inflection point of the titration curve (the point of intersection given by the extrapolation of two straight lines before and after the infection) as the end point in amperometric titration.

The blank test in this titration is usually performed as follows: Take a volume of the solvent specified in the individual monograph or in the "Standard Solution for Volumetric Analysis", and use this as the test solution. Determine the amount of the volumetric standard solution needed for giving the end point, and use this volume as the blank. If this volume is too small to determine accurately, the blank may be considered as 0 (mL).

Unless otherwise specified, the end point in this titration is decided by either of the following methods.

(i) *Drawing method* Usually, extrapolate the two straight lines before and after the infection, and obtain the inflection point of titration curve. Next, read the drop-wise quantity of titrant added at the infection point, and assume this point to be the end point.

(ii) *Automatic detection method* In the case of amperometric titration using an automatic titration system, the end point can be determined by following the instrumental indications. The end point is decided by following the variation of the indicator current during the course of a titration, and the drop-wise quantity of titrant added is assumed to be that at which the current has reached the end point current set previously for the individual titration system.

When atmospheric carbon dioxide or oxygen is expected to influence the titration, a beaker with a lid should be used, and the procedure should be carried out in a stream of an inert gas, such as nitrogen gas. Further, when a specimen is expected to be influenced by light, use a light-resistant container to avoid exposure of the specimen to direct sunlight.

18. Fats and Fatty Oils Test

The Fats and Fatty Oils Test is a method applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and related substances.

Preparation of test sample

For a solid sample, melt with care, and, if necessary, filter the melted sample with a dry filter paper by warming. For a turbid liquid sample, heat at about 50 °C. If it is still turbid, filter it with a dry filter paper while warm. In either case, mix the sample to make it homogeneous.

Melting point

Proceed by the method described in Method 2 of the Melting Point Determination.

Congealing point of fatty acids

(1) *Preparation of fatty acids* Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1000mL beaker, and heat at 150 °C. Add 50 g of the sample to this solution, and heat at a temperature not higher than 150 °C for 15 minutes under frequent stirring to saponify completely. Cool the solution to 100 °C, dissolve by addition of 500mL of hot water, and add slowly 50mL of diluted sulfuric acid (1 in 4). Heat the solution under frequent stirring until the clear layer of fatty acid is separated distinctly. Separate the fatty acid layer, and wash the fatty acid with hot water until the washing shows no acidity to methyl orange TS. Transfer the fatty acid phase to a small beaker, and heat on a water bath until the fatty acid becomes clear owing to the separation of water. Filter the warm solution, and complete the evaporation of water by carefully heating the filtered solution to 130 °C.

(2) *Measurement of congealing point* Proceed by the method described in the Congealing Point Determination.

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. Weigh accurately the pycnometer, and, after discarding the water and drying, weigh accurately the empty pycnometer. Then, fill the pycnometer with the melted sample to about three-fourths of the depth, and allow to stand at a temperature a little higher than the melting temperature of the sample for 1 hour to drive off the air in the sample. After keeping at the specified temperature, weigh accurately the pycnometer. Fill up the pycnometer with water over the sample at 20 °C, and weigh accurately again.

The other procedure is the same as described in Method 1 of the Determination of Specific Gravity and Density.

$$d = \frac{M_1 - M}{(M_2 - M) - (M_3 - M_1)}$$

M: Mass of the empty pycnometer (g)
M_1: Mass of the pycnometer filled with the sample (g)
M_2: Mass of the pycnometer filled with water (g)
M_3: Mass of the pycnometer filled with the sample and water (g)

Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids in 1 g of sample.

Procedure Unless otherwise specified, weigh accurately the amount of sample shown in Table 1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100mL of a mixture of diethyl ether and ethanol(95) (1:1 or 2:1) as the solvent, and dissolve the sample by warming, 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 which persists for 30 seconds. If the test solutions is turbid at lower temperature, titration should be done while warm. To the solvent used add phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS before use, until the solvent remains light red for 30 seconds.

$$\text{Acid value} = \frac{\text{consumed volume(mL) of } 0.1\,\text{mol/L potassium hydroxide} \cdot \text{ethanol VS} \times 5.611}{\text{amount(g) of sample}}$$

Table 1

Acid value	Amount (g) of sample
Less than 5	20
Not less than 5 and less than 15	10
Not less than 15 and less than 30	5
Not less than 30 and less than 100	2.5
Not less than 100	1.0

Saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and to neutralize the free acids in 1 g of the sample.

Procedure Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a short reflux condenser or an air condenser 750 mm in length and 6 mm in diameter to the neck of the flask, and heat gently in a water bath for 1 hour with frequent shaking. Cool the solution, add 1mL of phenolphthalein TS, and titrate immediately the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS. If the test solution is turbid at lower temperature, titration should be done while warm. Perform a blank determination.

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}}$$

a: Volume of 0.5 mol/L hydrochloric acid VS consumed in the blank determination (mL)
b: Volume of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample (mL)

Ester value

The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

Procedure Unless otherwise specified, designate the difference between the saponification value and the acid value determined as the ester value.

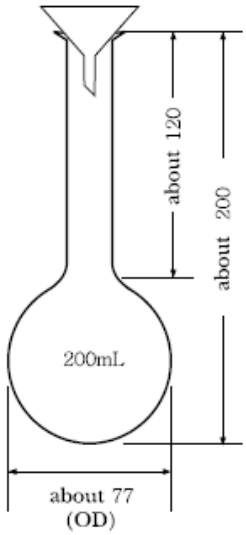
Hydroxyl value

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 Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in the figure), and add exactly 5mL of pyridine-acetic anhydride TS. Place a small funnel on the neck of the flask, and heat by immersing the flask up to 1 cm from the bottom in an oil bath between 95 °C and 100 °C. Put a thick, round paper with a round hole on the joint of the neck of the flask to protect the neck from the heat of the oil bath. After heating for 1 hour, take the flask from the oil bath, and cool by standing. Add 1mL of water to the flask, and shake to decompose acetic anhydride. Heat the flask in the oil bath for 10 minutes again. After cooling, wash the funnel and neck with 5mL of neutralized ethanol down into the flask, and titrate with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1mL of phenolphthalein TS). Perform a blank determination.

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{amount (g) of sample}} + \text{acid value}$$

a: Volume of 0.5 mol/L potassium hydroxide-ethanol VS consumed in the blank determination (mL).
b: Volume of 0.5 mol/L potassium hydroxide-ethanol VS consumed for titration of the sample (mL).



The figures are in mm.

Unsaponifiable matter

Unsaponifiable matter is calculated as the difference between the amount of materials, which are unsaponifable by the procedure described below, soluble in ether and insoluble in water, and the amount of fatty acids expressed in terms of the amount of oleic acid. Its limit is expressed as a percentage in the monograph.

Procedure Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100mL of warm water, and transfer the washing to the separator. Further, add 50mL of water to the separator, and cool to room temperature. Wash the flask with 100mL of diethyl ether, add the washing to the separator, extract by vigorous shaking for 1 minute, and allow to stand until both layers are separated clearly. Transfer the water layer to the second separator, add 50mL of diethyl ether, shake, and allow to stand in the same manner. Transfer the water layer in the second separator to the third separator, add 50mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and combine the washings into the first separator. Wash the combined extracts in the first separator with 30mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolpohthalein TS. Add a small amount of anhydrous sodium sulfate to the diethyl ether extracts, and allow to stand for 1 hour. Filter the diethyl ether extracts with dry filter paper, and collect the filtrates into a tared flask. Wash well the first separator with diethyl ether, and add the washing to the flask through the above filter paper. After evaporation of the filtrate and washing almost to dryness on a water bath, add 3mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70 °C and 80 °C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2mL of diethyl ether and 10mL of neutralized ethanol, and dissolve the residue by shaking well. 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 the solution develops a light red color which persists for 30 seconds.

$$\text{Unsafonifable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{amount (g) of sample}} \times 100$$

a: Amount of the extracts (g)

b: Volume of 0.1 mol/L potassium hydroxide-ethanol VS consumed for titration (mL).

Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

Procedure Unless otherwise specified, weigh accurately the amount of sample shown in Table 2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask, place the container containing the sample, add 20mL of cyclohexane to dissolve the sample, then add exactly 25mL of Wijs' TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20 °C and 30 °C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20mL of potassium iodide solution (1 in 10) and 100mL of water, and shake. Then, titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume of 0.1 mol/ L sodium thiosulfate VS consumed in the blank determination (mL)

b: Volume of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample (mL)

Table 2

Iodine value	Amount (g) of sample
Less than 30	1.0
Not less than 30 and less than 50	0.6
Not less than 50 and less than 100	0.3
Not less than 100	0.2

19. Flame Coloration Test

The Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

Salt of metal The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid test specimen, make the specimen into a gruel by adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid test specimen, immerse the end of the platinum wire into the specimen to about 5 mm in length, remove from the specimen gently, and perform the test in the same manner as for the solid sample.

Halide Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip, 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the test specimen to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, "Flame coloration persists", in a monograph, indicates that the reaction persists for 4 seconds.

20. Fluorometry

The Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an excitation light in a certain wavelength range. The Fluorometry is also applied to the phosphorescent substances.

Fluorescence intensity F in a dilute solution is proportional to the concentration c in mol per liter of the solution and the pathlength l of light through the solution in centimeter.

$$F = k \cdot P_0 \cdot \phi \cdot \varepsilon \cdot c \cdot l$$

k: Constant,

P_0 : Intensity of excitation light,

ϕ: Quantum yield of fluorescence or phosphorescence.

$$= \frac{A}{\text{number of quanta absorbed}}$$

A: Number of quanta emitted as fluorescence or phosphorescence

ε: Molar extinction coefficient of the substance at the excitation wavelength.

Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable excitation light are used as the light source. Usually, a non-fluorescent quartz cell (1 cm × 1 cm) with four transparent sides is used as the container for test solution.

Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of test solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of test solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement. Unless otherwise specified, the instrument is operated as follows with standard solution, test solution, and reference solution prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60 % to 80 % of full scale. Then perform the measurements with the cells containing the test solution and the reference solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

Note: The fluorescence intensity is readily affected by the concentration, temperature, and pH of the solution, and nature and purity of solvents or reagents used.

21. Foreign Insoluble Matter Test

The Foreign Insoluble Matter Test is a test method to examine foreign insoluble matters in ophthalmic solutions and injections.

Ophthalmic Solutions

Ophthalmic Solutions prepared as aqueous solution and aqueous vehicles attached to the Ophthalmic Solutions to be prepared before use should be clear and free from foreign insoluble matter when inspected with the unaided eye at a position of luminous intensity of 3000 to 5000 luxes under an incandescent lamp.

Injections

Method 1. This method is applied to injections in solutions, and vehicles attached to the injections to be prepared before use.

Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 luxes under an incandescent lamp: Injections must be clear and free from readily detectable foreign insoluble matters. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity of approximately 8000 to 10,000 luxes, with incandescent lamps at appropriate distances above and below the container.

Method 2. This method is applied to injections with constituting solution.

Clean the exterior of containers, and dissolve the contents with constituting solution or with water for injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be clear and free from foreign insoluble matters that are clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 luxes, right under an incandescent lamp.

22. Gas Chromatography

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column (t_0: time measured from the time of injection of a compound with $k=0$ to the time of elution at the peak maximum), and the retention time (t_R: time measured from the time of in injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1+k)\,t_0$$

Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector, and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system to keep the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the test solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram.

Identification and purity test

Identification of a component of a test solution is performed by confirming agreement of the retention time of the component with that of an authentic specimen, or by confirming that the peak shape of the component is not changed after mixing the test solution with an authentic specimen.

In general, the purity of the sample is determined by comparing the test solution with a standard solution which is prepared by diluting the test solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate result in evaluating the proportion of the components, it is necessary to correct the areas of each component based on its response factor to the principal component.

Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

(1) ***Internal standard method*** In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. 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 curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a test solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

(2) ***Absolute calibration curve method*** Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a test solution according to the method specified in the individual monograph, perform the gas chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a test solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

(3) ***Standard addition method*** Pipet a fixed volume of more than 4 test solutions, add exactly the standard solution so that step-wise increasing amounts of the object compound are contained in the solutions except 1 test solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the test solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual test solutions, measure the peak area or peak height of individual test solutions. Calculate the concentration of standard objective compound added into each test solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area or peak height on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

Method for peak measurement

Generally, the following methods are used.

(1) *Peak height measuring method*

(i) *Peak height method:* Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) *Automatic peak height method*: Measure the signals from the detector as the peak height using a data processing system.

(2) *Peak area measuring method*

(i) *Width* at *half-height method*: Multiply the peak width at the half-height by the peak height.

(ii) Automatic *integration method*: Measure the signals from the detector as the peak area using a data processing system.

System suitability

System suitability testing is an integral part of test methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. System suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test method of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

In system suitability testing of the chromatographic systems, the evaluation of "System performance" and "System repeatability" is usually required. For quantitative purity test, the evaluation of "Test for required detectability" may also be required.

(1) *Test for required detectability*

For purity tests, when it is confirmed that the target impurity is distinctly detected at the concentration of its specification limit, it is considered verified that the system used has adequate performance to achieve its intended use.

For quantitative purity test, "Test for required detectability" is usually required, and in order to confirm, in some degree, the linearity of response near its specification limit, the range of expected response to the injection of a certain volume of target impurity solution at the concentration of its specification limit should be prescribed. For limit test, "Test for required detectability" is not required, if the test is performed by comparing the response from sample solution with that from standard solution at the concentration of its specification limit. "Test for required detectability" is also not required, if it is confirmed that the impurity can be detected at its specification limit by the evaluation of "System repeatability" or some other procedure.

(2) *System performance*

When it is confirmed that the specificity for determining the test ingredient is ensured, it is considered verified that the system used has adequate performance to achieve its intended use.

In assay, "System performance" should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable), and when appropriate, by their order of elution. In purity tests, both the resolution and the order of elution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable) should be prescribed. In addition, if necessary, the symmetry factor if the test ingredient should be prescribed together with them. However, if there is no suitable target substance to be separated, it is acceptable to define "System performance" using the number of theoretical plates and the symmetry factor of the test ingredient.

(3) *System repeatability*

When it is confirmed that the degree of variation (precision) of the response of the test ingredient is at a level that meets the requirement of "System repeatability", it is considered verified that the system used has adequate performance to achieve its intended use.

The allowable limit of "System repeatability" is normally defined as the relative standard deviation (RSD) of the response of the test ingredient in replicate injections of standard solution. It is acceptable to confirm the repeatability of the system not only by replicate injections of standard solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of "System repeatability" which can guarantee a level of "System repeatability" equivalent to that at 6 replicate injections.

The allowable limit of "System repeatability" should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

Point to consider on changing the operation conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the column packing material, concentration of the stationary phase, column temperature, and flow rate of carrier gas may be modified within limits which allow the required elution order, resolution, symmetry factor and relative standard deviation to be obtained. The sample injection port and the operating conditions for headspace may be also modified within limits which allow the accuracy and precision more than those of a prescribed method to be obtained.

Terminology

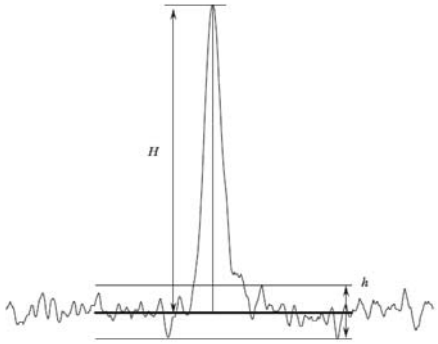
(1) *SN ratio* : It is defined by the following formula.

$$S/N = \frac{2H}{h}$$

H : peak height of the target ingredient peak from the baseline (the median value of background noise)

h : Width of background noise of the chromatogram of sample solution or solvent blank around the peak of the target ingredient

The baseline and background noise are measured over a range 20 times of peak width at the center point of peak height of the target ingredient. When a solvent blank is used, measure over almost the same range as mentioned above around the point where the target ingredient.



(2) *Symmetry factor* Symmetry factor shows the degree of symmetry of a peak in the chromatogram, and is defined as Sin the following equation.

$$S = \frac{W_{0.05\,\mathrm{h}}}{2f}$$

$W_{0.05\mathrm{h}}$: Width of the peak at one-twentieth of the peak height.

f : Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height, where $W_{0.05\mathrm{h}}$ and f have the same unit.

(3) *Relative standard deviation* Generally, it is given as RSD(%) defined by the following equation.

$$S_{\mathrm{R}}(\%) = \frac{100}{X} \times \sqrt{\frac{\sum_{i=1}^{n}(x_i - X)^2}{n-1}}$$

x_i : Measured value,

$\overline{X}$: Mean of measured values, and

n : Number of repeated measurements.

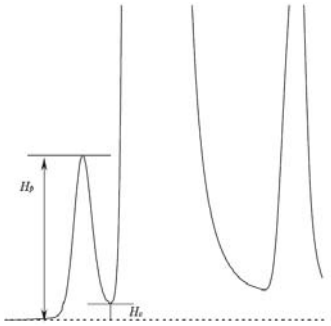
(4) *Complete separation of peak* Complete separation of the peak means that the resolution between two peaks is not less than 1.5.

(5) *Peak-valley* ratio It indicates the degree of separation between 2 peaks on a chromatogram when baseline separation cannot be attained, and 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.



(6) *Separation factor* Separation factor shows the relation between the retention times of peaks in the chromatogram, and is defined as α in the following equation. The separation factor (α) is a characteristic of the indicates thermodynamic difference in partition of two compounds. It is basically the ratio of their partition equilibrium coefficients or of their mass-distribution ratios, and is obtained from the chromatogram as the ratio of the retention times of the two compounds.

$$\alpha = \frac{(t_{\mathrm{R}2} - t_0)}{(t_{\mathrm{R}1} - t_0)}$$

$t_{\mathrm{R}1}$, $t_{\mathrm{R}2}$: Retention times of two compounds used for the resolution measurement($t_{\mathrm{R}1} < t_{\mathrm{R}2}$), and

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).

(7) *Resolution* Resolution shows the relation between the retention time and the peak width of peaks in the chromatogram, and is defined as R_s Rsin the following equation.

$$R_S = 1.18 \times \frac{(t_{\mathrm{R}2} - t_{\mathrm{R}1})}{(W_{0.5\,\mathrm{h}1} + W_{0.5\,\mathrm{h}2})}$$

$t_{\mathrm{R}1}$, $t_{\mathrm{R}2}$: Retention times of two compounds used for measurement of the resolution ($t_{\mathrm{R}1} < t_{\mathrm{R}2}$), and

$W_{0.5\mathrm{h}1}$, $W_{0.5\mathrm{h}2}$: Peak widths at half peak height, where $t_{\mathrm{R}1}$, $t_{\mathrm{R}2}$, $W_{0.5\mathrm{h}1}$, $W_{0.5\mathrm{h}2}$ the same unit.

(8) ***Number of theoretical plates*** Number of theoretical plates is generally defined in terms of the as N in the following equation to indicate the extent of the band broadening of a compound in the column.

$$N = 5.55 \times \frac{{t_R}^2}{{W_{0.5\,h}}^2}$$

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 Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

23. Heavy Metals Limit Test

The Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with sodium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

(1) ***Method 1*** Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40mL. Add 2mL of dilute acetic acid and water to make 50mL, and use this solution as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2mL of dilute acetic acid and water to make 50mL.

(2) ***Method 2*** Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500 °C and 600 °C. Cool, add 2mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2mL of dilute acetic acid, filter if necessary, and wash with 10mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50mL. Use this solution as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2mL of nitric acid, 5 drops of sulfuric acid, and 2mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50mL.

(3) ***Method 3*** Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then incinerate by ignition between 500 °C and 600 °C. After cooling, add 1mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2mL of dilute acetic acid, filter if necessary, wash with 10mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50mL. Use this solution as the test solution.

The control solution is prepared as follows: Evaporate 1mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50mL.

(4) ***Method 4*** Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1mL of sulfuric acid, heat carefully, and incinerate by ignition between 500 °C and 600 °C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2mL of dilute acetic acid, filter if necessary, wash with 10mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and fire the ethanol to burn. Cool, add 1mL of sulfuric acid, heat carefully, and ignite between 500 °C and 600 °C. Cool, and add 3mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50mL.

(5) ***Method 5*** Unless otherwise specified in the monograph, place 0.3 g of extract or 1.0 g of fluidextract in a platinum or porcelain crucible, evaporate to dryness on a water bath, incinerate by ignition between 500 °C and 600 °C. Cool, dissolve the residue in 3mL of hydrochloric acid by warming, filter and wash the residue with 5mL of water two times. Transfer the filtrate and washings to a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a

pale red color develops, then add 2mL of dilute acetic acid, and add water to make 50mL. Use this solution as the test solution.

The control solution is prepared as follows: Add 3mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add 3.0mL of Standard Lead Solution and water to make 50mL.

Procedure

Add 1 drop of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solutions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

24. Infrared Spectrophotometry

The Infrared Spectrophotometry is a method of measurement of the extent, at various wavenumbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wavenumbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used be identify or determine a substance.

lnstrument and adjustment

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for the resolving power, transmittance reproducibility, and wavenumber reproducibility. When the spectrum of a polystyrene film about approximately 0.04 mm thick is recorded, the depth of the trough from maximum absorption at about 2851 cm^{-1} to the minimum at about 2870 cm^{-1} is not less than 18 % transmittance and that from the maximum at about 1583 cm^{-1} to the minimum at about 1589 cm^{-1} is not less than 12 % transmittance.

The wave number scale is usually calibrated by the use of specified wave numbers (cm^{-1}) of a polystyrene film, shown below. Also, the numbers in parentheses indicate the approved range with which these values have been established.

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)		

The approval range in the case of used dispersion apparatus 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}.

The difference of transmittance is within 0.5 % when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm^{-1} and the difference of wavenumber should be within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1}.

Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures so that the transmittance of most absorption bands is to be in the range of 5 % to 80 %. Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate. Generally, the reference cell or materials placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between 4000 cm^{-1} and 400 cm^{-1}. The spectrum is scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale, and of wave numbers.

(1) ***Potassium bromide disk or potassium chloride disk method*** Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide or potassium chloride used to infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. If necessary to obtain a transparent disk, press the mixture under vacuum within 0.67 kPa in a die with pressure applied to the die of 50 to 100 kN per cm^2 for 5 to 8 minutes. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk.

(2) ***Solution method*** Place the test solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the test solution. The solvent used in this method does not show any interaction or chemical reaction with the specimen to be examined and does not 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 specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 or 2 drops of

liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

(4) ***Liquid film method*** Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

(5) ***Film method:*** Examine a thin film just as it is or a prepared thin film as directed in each monograph.

(6) ***Gas sampling method*** Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

(7) ***ATR method:*** Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

(8) ***Diffuse reflectance method*** Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about 50 μm particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride used to infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

Identification

(1) ***Identification by the use of reference standard*** When the spectra of a specimen and the reference standard exhibit similar intensities of absorption at the same wavenumber, the specimen can be identified as being the same substance as the reference standard. When several specific absorption wavenumbers of the substance being examined are specified in the monograph, the specimen and reference standard are same substance if wavenumbers of two substances are coincide. When the spectra of a specimen and the reference standard exhibit similar intensities of absorption at the same wavenumber, the specimen can be identified as being the same substance as the reference standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the reference standard, treat the specimen being examined and the reference standard in the same manner as directed in the monograph, then repeat the measurement.

(2) ***Identification by the use of absorption wave number*** When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

25. Insoluble Particulate Matter Test for Injections

The insoluble particulate matter test for injections or infusions is the method to test for insoluble particulates, to confirm that they are not present in excess of specified levels in the solutions. For the determination of particulate contamination, 2 procedures, Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test), are specified hereinafter. Method 1 is preferably applied. However, it may be necessary to test some preparations by Method 2 followed by Method 1 to reach a conclusion on conformance to the requirements. Not all injections can be examined by one or both of these methods. When Method 1 is not applicable, e.g. in case of preparations having reduced clarity or increased viscosity, emulsions, colloids, liposomal preparations, and products that produce air or gas bubbles when drawn into the sensor, the test is carried out according to Method 2. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results are obtained by examining a discrete unit or group of units. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterize the level of particulate contamination in a large group of units.

Method 1. Light Obscuration Particle Count Test

(i) ***Apparatus*** Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year.

(ii) ***Calibration*** Particles to be used for calibration should be subject to particle size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25 mm in diameter (PSL particles) in mono-dispersed suspension. The PSL particles have either a domestic or international traceability in terms of length, with a level of uncertainly at not greater than 3 %. The particles to be used for calibration should be dispersed in particle-free water.

(iii) ***Manual method*** The particle size response of the system to be applied 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 size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring

point, and threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained.

(iv) ***Electronic method*** In the use of multichannel peak height analyzer, the particle size response is measured by half-count method of moving window system same as the manual method, and the particle size response curve is prepared by the method designated by the instrument manufacturer, then, the threshold voltage of 5, 10 and 25 μm of the apparatus is obtained. In this case, the obtainability of the same result as that of the manual method is validated.

(v) ***Automated method*** The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas, the obtainability of the same result as that of the manual method is validated.

(vi) ***Sample volume accuracy*** Sample volume accuracy falls within 5 % of the measuring value in case measuring the decrease of test solution by the mass method after measuring the test solution of 10mL.

(vii) ***Sample flow rate*** The flow rate of the sample indicated into the sensor is calculated from the observed sample volume and time, and is conformed within the range of the manufacturer's specification for sensor used.

(viii) ***Sensor counting rate accuracy*** There is a possibility of changes of particle size resolution and counting rate of particle detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same-type sensor. The sensor counting rate accuracy also needs to be confirmed. Testing is accordingly performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PS spheres having mean diameter of approximately 10 μm, of a concentration at 1000 particles/mL ± 10 %, relative standard deviation of not more than 5 %). During measurement, stirring is made for assuring the uniformity in sample.

(ix) ***Sensor resolution*** (particle size resolution of apparatus) Measurement is made by either one of the following methods: a) manual method to obtain the spread of histogram prepared from the counting value of the apparatus b) electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer c) automated method to obtain the spread of histogram of responsive signal of the test-particle by using the software prepared by the manufacturer or the user.

The difference between the threshold of particle size counting 16 % and 84 % of the total counts and the test-particle size is within 10 %, whereas, electronic method and automated method is both validated for obtaining the same result as that of the manual method.

(x) ***Particle counting accuracy*** Data obtained by counting particles of not less than 5 mm is 763 to 1155 particles permL.

(xi) ***Threshold accuracy*** Particle size calculated from a threshold corresponding to 50 % counts for particles of 5 μm and greater falls within ± 5 % of the mean diameter of the test particles.

(xii) ***General precautions*** The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet. Wash very carefully the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with particle-free water. Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out. In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of particle-free water, each of 5mL, according to the method described below. If the number of particles of 10 μm or greater in size exceeds 25 for the combined 25mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

(xiii) ***Procedure*** Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 minutes or sonicating.

For injections having a volume of 25mL or more, not more than 10 units may be tested, based on an appropriate sampling plan.

For small-volume injections less than 25mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25mL: where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25mL with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

Powders for injection are reconstituted with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

Remove 4 portions, each of not less than 5mL, and count the number of particles equal to or greater than 10 μm and 25 μm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

(xiv) ***Evaluation*** If the average number of particles exceeds the limits, test the preparation by Method 2 (Microscopic Particle Count Test).

a) Injections with a nominal volume of not less than than 100mL : The preparation complies with the test if the mean number of particles present in the units

tested is not more than 25 permL for particles with size of not less than 10 μm and not more than 3 permL for particles with size of not less than 25 μm.

b) Injections with a nominal volume of less than 100mL : The preparation complies wiih the test if the average number of particles present in the containers tested is not more than 6000 per container for particles with size of not less than 10 μm and is not more than 600 per container for particles with size of not less than 25 μm.

Method 2. Microscopic Particle Count Test

(i) ***Apparatus*** Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination. The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, and illuminators and is adjusted to 100 ± 10 magnifications. The ocular micrometer is a circular diameter graticule (see Fig. 1) and consists of a large circle designated as the graticule field of view (GFOv), divided by crosshairs into quadrants transparent and black reference circles 10 μm and 25 μm in diameter at 100 magnifications, and a linear scale graduated in 10 μm increments. It is calibrated using a stage micrometer that is certified by either a domestic or international standard institution. A relative error of the linear scale of the graticule within ± 2 % is acceptable.

(ii) ***Illuminators*** Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, and the other is an external, focussable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10˚ to 20˚.

(iii) ***Filter assembly*** The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter. The membrane filter is of suitable size, black or dark gray in color, non-gridded or gridded, and 1.0 μm or finer in nominal pore size.

(iv) ***General precautions*** The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet. Wash very carefully the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with particle-free water. In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 5mL volume of particle-free water according to the method described below. If more than 25 particles of not less than 10 μm in size are present in 25mL, measured 5 times, the environment for the test are not suitable. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

(v) ***Procedure*** Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water and remove the closure, avoiding any contamination of the contents.

For injections not less than 25mL in volume, single uinits are tested. For injections less than 25mL in volume, the contents of not less than 10 units are combined in a cleaned container, where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25mL with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

Powders for injection are reconstituted with particle-free water or with an appropriate solvent without contamination of particles when particle-free water is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For injections having volume of not less than 25mL, not more than 10 containers may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliters of particle-free water. Transfer to the filtration funnel the total volume of a solution pool or of a single container, and apply vacuum to filter. After the filtration, begin rinsing the inner walls of the filter holder by using a jet of particle-free water. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are not less than 10 μm and the number of particles that are not less than 25 μm. Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined. The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the image of each particle into a circle and then comparing it to the 10 μm and 25 μm graticule reference circles. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test (Method 2) do not attempt to size or enumerate amorphous, semi-liquid, or otherwise morphologically indistinct materials that have the appearance of a stain or

discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by Method 1.

(vi) ***Evaluation***

a) Injections with a nominal volume of not less than 100mL: The preparation complies with the test if the mean number of particles present in the units tested does not exceed 12 permL for with size of not less than 10 μm and not more than 2 permL for particles with size of not less than 25 μm.

b) Injections with a nominal volume of less than 100mL: The preparation complies with the test if the average number of particles present in the containers tested is not more than 3000 per container for particles with size of not less than 10 μm and is not more than 300 per container for particles with size of not less than 25 μm.

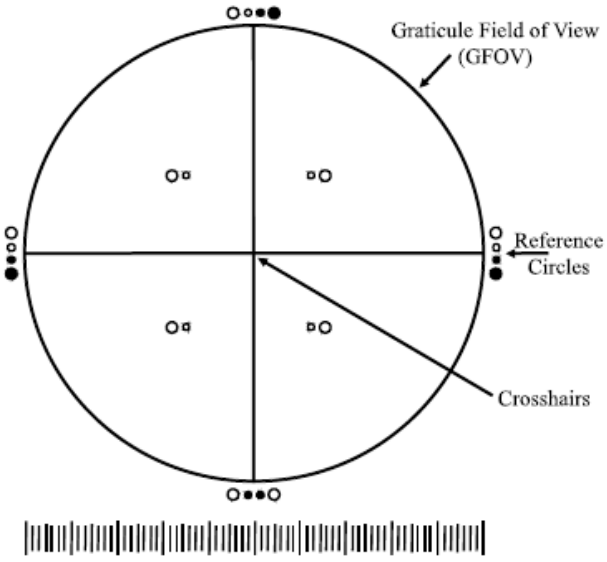


Fig. 1 Circular diameter graticule

Reagents Particle-free water: The filtered water through a membrane filter with a pore size not exceeding 0.45 μm, containing not more than 5 particles of 10 μm or greater size, and not more than 2 particles of 25 μm or greater size in 10mL of the insoluble particle number measured by light obscuration particle counter.

26. Insoluble Particulate Matter Test for Ophthalmic Solutions

Insoluble Particulate Matter Test for Ophthalmic Solutions is to examine for the size and the number of insoluble particulate matter in Ophthalmic Solutions.

Apparatus Apparatus consists of a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for determination.

(i) ***Microscope*** The microscope is equipped with a micrometer system, a mobile stage and an illuminator, and is adjusted to 100 magnifications.

(ii) ***Filter assembly for retaining insoluble particulate matter*** The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material causing no problem in testing, and a clip. The unit is capable of fitting with a membrane filter of 24 mm or 13 mm in diameter and can be used under reduced pressure.

(iii) ***Membrane filter for measurement*** The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter equal to or greater than 25 μm in size should not be found on the filter. When 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 not exceeding 0.45 μm. It contains not more than 10 particles of 10 μm or grater size in 100mL.

Procedure

(i) ***Aqueous ophthalmic solutions*** All operations are carried out carefully in clean facilities or equipment which are low in dust. The membrane filter is fit onto the membrane filter holder, and fixed with the clip. The water for particulate matter test is used to rinse the inside of the holder and is filtered through under reduced pressure with 200mL of the water for particulate matter test at a rate of 20 to 30mL per minute. The vacuum is applied until the surface of the membrane filter is free from water and the membrane filter is removed. The filter is placed in a flat-bottomed Petri dish with the cover slightly ajar and dried fully at a temperature not exceeding 50 °C. After the filter has been dried, the Petridish is placed on the stage of the microscope. Under a down-light from an illuminating device, the grid of the membrane filter is aligned to the coordinate axes of the microscope and the microscope is adjusted so as to get the best view of the insoluble particulate matter, then the number of the particles, of which diameter is not less than 150 μm, within the effective filtering area of the filter is counted, moving the mobile stage and ascertaining that the number is not more than 1. The particle is sized on the longest axis. Another membrane filter is fit to the filtration device, and fixed with the clip, and then the inside of the filter holder is wet with severalmL of water for particulate matter test. The outer surface of the sample container is cleaned and the test solution is mixed gently by inverting the container several times. The sample container is opened and the outer surface of the nozzle is cleaned. The test solution is poured into a measuring cylinder

which has been rinsed well with water for particulate matter test. The process is repeated to prepare 25mL of the test solution. The test solution is poured into the filter holder along the inner wall of the holder. The vacuum is applied for mild filtration so as to keep the solution always on the filter. Viscous test solution is diluted appropriately with water for particulate matter test. or suitable diluent and filtered in the same manner mentioned above. When the amount of the solution on the filter becomes small, 30mL of water for particulate matter test or suitable diluent is added in such a manner as to wash the inner wall of the filter holder. This process is repeated 3 times with 30mL of the water. The vacuum is applied gently until the surface of the membrane filter is free from water. The filter is placed in a petri dish with the cover slightly ajar and dried fully at a temperature not exceeding 50 °C. After the filter has been dried, the petri dish is placed on the stage of the microscope. The number of the particles, of which diameter is not less than 300 μm, within the effective filtering area of the filter is counted by the same procedure of the microscope as described above. The particle is sized on the longest axis.

(ii) ***Ophthalmic solutions dissolved before use*** The procedure is as directed in Aqueous ophthalmic solutions after dissolving the sample with constituted solution. The sample size is 25mL.

(iii) ***Suspension type ophthalmic solutions*** The procedure is as directed in Aqueous ophthalmic solutions. 25mL of the sample is taken in a vessel, which has been rinsed well with water for particulate matter test, and a suitable amount of a suspension-solubilizing solvent or an adequate solvent is added and shaken to dissolve the suspending particles, and this solution is used as the test solution. A membrane filter which is not affected by the solvent is used.

(iv) ***Single-dose ophthalmic solutions*** The procedure is as directed in aqueous ophthalmic solutions using 10 samples for the test. A membrane of 13-mm diameter and a filter holder of 4-mm diameter for retaining insoluble particulate matter are used.

Interpretation The limit of insoluble particulate matters is not more than 1 particulate of the diameter not less than 300 μm in 1mL of the solution.

27. Iron Limit Test

The Iron Limit Test is a limit test for iron contained in drugs. The limit is expressed in term of iron (Fe).

In each monograph, the limit for iron (as Fe) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, the test solutions and the control solutions are prepared as follows:

(1) ***Method 1*** Weigh the amount of sample specified in individual monograph, add 30mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, dissolve by warming, if necessary, and use this solution as the test solution.

Prepare the control solution as follows: To the amount of standard iron solution specified in individual monograph, add 30mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5.

(2) ***Method 2*** Weigh the amount of sample specified in individual monograph, add 10mL of dilute hydrochloric acid, and dissolve by warming, if necessary. Dissolve 0.5 g of L-tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS drop-wise until the solution develops a pale red color. Add 20mL of acetic acid-sodium acetate butter solution for iron limit test, pH 4.5, and use this solution as the test solution.

Prepare the control solution as follows: To the amount of standard iron solution specified in individual monograph, add 10mL of dilute hydrochloric acid, and proceed as directed for the test solution.

(3) ***Method 3*** Place the amount of sample specified in individual monograph in a crucible, moisten with a small amount of sulfuric acid, heat cautiously and gently at first, and then incinerate by ignition. After cooling, add 1mL of diluted hydrochloric acid (2 in 3) and 0.5mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue, add 0.5mL of diluted hydrochloric acid (2 in 3) and 10mL of water. After dissolving by warming, add 30mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and use this solution as the test solution.

Prepare the control solution as follows: Transfer the amount of standard iron solution specified in individual monograph to a crucible, and add 1mL of diluted hydrochloric acid (2 in 3) and 0.5mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and proceed as directed for the test solution. In this procedure, use a quartz or porcelain crucible, which is immersed in boiling dilute hydrochloric acid for 1 hour and washed thoroughly with water and dried.

Procedure

Unless otherwise specified, proceed as follows:

(1) ***Method A*** Transfer the test solution and the control solution to separate Nessler tubes, to each add 2mL of L-ascorbic acid solution (1 in 100), mix well, and allow to stand for 30 minutes. Add1mL of a solution of α,α'-dipyridyl in ethanol (1 in 200), add water to make 50mL, and allow to stand for 30 minutes. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

(2) ***Method B*** Dissolve 0.2 g of ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1mL of a solution of a,a'-dipyridyl in ethanol (1 in 200), and allow to stand for 30 minutes. Then add 2mL of a solution of picric acid (3 in 1000) and 20mL of 1,2-dichloroethane, shake vigorously, collect the 1,2-dichloroethane layer, and filter through a pledget of absorbent cotton in a funnel on which 5 g of anhydrous sodium sulfate is placed, if necessary. Then compare the colors developed in both

solutions against a white background. The test solution has no more color than the control solution.

28. Liquid Chromatography

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and assay. A mixture injected into a column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

The ratio k represents the mass distribution ratio(or the capacity factor) k' in liquid chromatography.

Since the relation given below exists among the ratio (k), the time for which the mobile phase Is passed through the column (t_0 : time measured from the time of injection of a compound with k=0 to the time of elution at the peak maximum), and the retention time (t_0 : time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k)\, t_0$$

Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector, and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few g. The recorder is used to record the output signals of the detector. As required, a data process or may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a step-wise or gradient fashion.

Procedure

Fix the detector, column, and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the test solution or the standard solution specified in the individual monograph with the sample injector into the column through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

Identification and purity test

When Liquid Chromatography is used for identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sample with an authentic specimen.

If a detector which is able to obtain chemical structural information of the component at the same time is used, more specific identification can be achieved by confirming identity of the chemical structure for the component and their retention times.

When this method is used for purity test, generally the purity of the sample is determined by comparing the test solution with the standard solution which is prepared by diluting the test solution to a concentration corresponding to the specified limit of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on the sensitivity factor to the principal component.

Assay

(1) ***Internal standard method*** In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. 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 curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a test solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

(2) ***Absolute calibration curve method*** Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a test solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a test solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

Method for peak measurement

The method for peak measurement under Gas Chromatography shall apply in the Liquid Chromatograpy.

System suitability

The System suitability under Gas Chromatography shall apply in the Liquid Chromatograpy.

Point to consider on changing the operation conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column temperature, composition ratio of the mobile phase, pH of the mobile phase, composition of the buffer solutions in the mobile phase, concentration of ion pair-forming agents in the mobile phase, ionic strength of the mobile phase, numbers of condition changes, timing of such changes, gradient program, composition and flow rate of derivative-producing reagents, reaction time and temperature of reaction chamber and flow rate of mobile phase may be modified within limits which allow the required elution order, resolution, symmetry factor, and relative standard deviation to be obtained.

Terminology

The definition of terms described under the Gas Chromatography shall apply in the Liquid Chromatography.

Note

Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

29. Loss on Drying Test

The Loss on Drying Test is a method to measure the loss in mass of the sample, when dried under the conditions specified in each monograph. This method is applied to determine the amount of water, all or a part of water of crystallization, or volatile matter in the sample, which is removed during the drying.

The description, for example, "not more than 1.0 percent (1 g, 105 °C, 4 hours)" in a monograph, indicates that the loss in mass is not more than 10 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed and dried at 105 °C for 4 hours, and "not more than 0.5 percent (1 g, in vacuum, phosphorus (V) oxide, 4 hours)," indicates that the loss in mass is not more than 5 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed, transferred into a desiccator (phosphorus (V) oxide), and dried in vacuum for 4 hours.

Procedure

Weigh accurately a weighing bottle that has been dried for 30 minutes according to the method specified in the monograph. Take the sample within the range of ±10 % of the amount directed in the monograph, transfer into the weighing bottle, and, unless otherwise specified, spread the specimen so that the layer is not thicker than 5 mm, then weigh it accurately. Place the loaded bottle in a drying chamber, and dry under the conditions specified in the monograph. When the size of the sample is large, convert it to small particles having a size not larger than 2 mm in diameter by quick crushing, and use the crushed sample for the test. After drying, remove from the drying chamber, and reweigh accurately. When the sample is dried by heating, the temperature is within the range of ±2 °C of that directed in the monograph, and, after drying the bottle, the specimen is allowed to cool in a desiccator (silica gel) before weighing.

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 and 10 °C below the melting temperature, dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and renew frequently.

30. Loss on Ignition Test

The Loss on Ignition Test is a method to measure the loss in mass when the sample is ignited under the conditions specified in each monograph. This method is usually applied to inorganic drugs which lose a part of the components or impurities during ignition.

The description, for example, "40.0 ~ 52.0 % (1 g, 450 ~ 550 °C, 3 hours)" in a monograph, indicates that the loss in mass is 400 mg to 520 mg per g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited between 450 °C and 550 °C for 3 hours.

Procedure

Previously ignite a crucible or a dish of platinum, quartz or porcelain to a constant mass, at the temperature directed in the monograph, and weigh accurately after cooling.

Take the sample within the range of ± 10 % of the amount directed in the monograph, transfer into the above ignited container, and weigh it accurately. Ignite under the conditions directed in the monograph, and after cooling by standing in a desiccator (silica gel), reweigh accurately.

31. Melting Point Determination

The Melting Point is defined to be the temperature at which a crystalline substance melts during heating, when the solid phase and the liquid phase are in an equilibrium. However, in this Pharmacopoeia it is conventionally defined to be the temperature at which the remaining solid sample melts completely when it is subjected to continuous heating and the change of the sample state that accompanies heating is accurately observed.

Since a pure substances has an intrinsic melting point, it is used for the identification and/or confirmation of a substance and also as an indicator of the purity of a substance.

The melting point is determined by either of the following methods:

Method 1 is applied to those substances of which the purity is comparably high and which can be pulverized, Method 2 to those substances which are insoluble in water and cannot be readily pulverized, and Method 3 to petrolatums. Unless otherwise specified, measurement is performed by Method 1.

Method 1

This method is applied to those substances of which the purity is comparably high and which can be purlverized.

(1) Apparatus Use the apparatus illustrated in the figure.

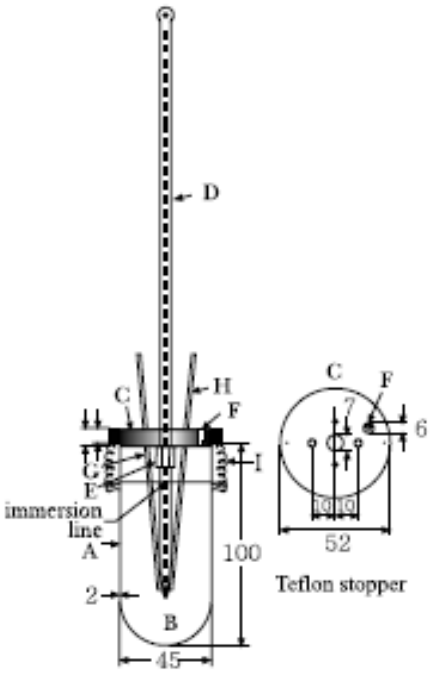
Alternatively, apparatus in which some of the procedures, such as stirring, heating, and cooling, are automated can be used.

Bath fluid: Use clear silicone oil having the viscosity of 50 to 100 mm2/s at ordinary temperature.

Thermometer with an immersion line: There are six types of thermometers, Type 1 –Type 6, which are specified by an appropriate measuring temperature range. For melting points lower than 50 °C, use a thermometer

Type 1; for 40 °C to 100 °C, Type 2; for 90 °C to 150 °C, Type 3; for 140 °C to 200 °C, Type 4; for 190 °C to 250 °C, Type 5; for 240 °C to 320 °C, Type 6.

Capillary tube: Use a hard glass capillary tube 120 mm long, 0.8 mm to 1.2 mm in inner diameter and with walls, 0.2 mm to 0.3 mm thick; one end is closed.



A: Heating receptacle (hard glass)
B: Bath fluid
C: Teflon stopper
D: Thermometer with an immersion line
E: Thermometer-fastening spring
F: Vent for adjustment of the bath fluid volume
G: Coil spring
H: Capillary Tube
I: Spring for fastening the stopper

(2) ***Procedure*** Reduce the sample to a fine powder, and, unless otherwise specified, dry in a desiccator (silica gel) for 24 hours. When it is specified to do the test after drying, dry the sample under the conditions in the test for Loss on Drying before the determination. Place the sample in a dried capillary tube H, and pack the sample tightly in a layer about 3 mm in height by dropping it repeatedly, with the closed end of H down, through a glass tube, about 70 cm long, held vertically on a glass or porous plate. Heat the bath fluid B until the temperature rises to about 10 °C below the expected melting point, place the immersion line of the thermometer D at the same level as the meniscus of the bath fluid, and insert capillary tube H into coil spring G so that the sample is on a level with the middle of the mercury bulb of the thermometer D. Then continue the heating to raise the temperature at a rate of approximately 3 °C per minute until the temperature rises to 5 °C below the expected 1296 General Tests, Processes and Apparatus melting point, then carefully regulate the rate of increase to 1 °C per minute. Read the indication of the thermometer D at the point at which the sample liquefies throughout and no solid is visible in the capillary tube H, and designate the temperature as the melting point.

System suitability test—Conformation of the system suitability of the apparatus should be done periodically by using the Melting Point Reference Standards. The Reference Standard is prepared for the suitability test of the apparatus when it is used with Type 2 –Type 5 thermometers, and consists of 6 highly purified substances: acetanilide, acetophenetidine, caffeine, sulfanilamide, sulfapyridine, and vanillin. The label shows the certified melting points of the respective substances (the end point of the melting change), MP_f.

After selecting one of the thermometers and the appropriate Melting Point Reference Standard based upon the expected melting point of a sample specimen, perform a melting point measurement of the selected Reference Standard, according to the above procedure.

When the value of the obtained melting point of the Reference Standard is within $MP_f \pm 0.5$ °C in the case of vaniline and acetanilide, within $MP_f \pm 0.8$ °C in the case of acetophenetidine and sulfanilamide, and within $MP_f \pm 1.0$ °C in the case of sulfapyridine and caffeine, the apparatus is assumed to be suitable.

The above-mentioned measurement is repeated 3 times and the average is determined to be the melting point of the Reference Standard tested. When the above suitability test criteria are not met in a certain melting point measurement system of an apparatus and a Reference Standard, do the test again, after checking the packing of the sample specimen into the capillary tube, the locations and positioning of the thermometer and the capillary tube, the heating and stirring of the bath fluid, and the control of the temperature increasing rate.

When a melting point measurement system does not meet the suitability test criteria again after checking these measuring conditions, the thermometer with an immersion line should be calibrated again or replaced with a new one.

Method 2

This method is applied to such substances as fats, fatty acids, paraffins or waxes.

(1) ***Apparatus*** Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line. Furthermore, the capillary tube should be the same as specified in Method 1, except that both ends of the tube are open.

(2) ***Procedure*** Carefully melt the sample at as low a temperature as possible, and, taking care to prevent bubbles, draw it into a capillary tube (one as used in Method 1 and which is left open at both ends) to a depth of about 10 mm. Allow the charged tube to stand for 24 hours at a temperature below 10 °C, or for at least 1 hour in contact with ice, holding the tube so as not to allow loss of the sample from it. Then attach the tube to the thermometer by means of a rubber band so that the sample is on a level with the middle part of the mercury bulb. Adjust the tube on a water- containing beaker to such a position that the lower edge of the sample is 30 mm below the water surface. Heat the

beaker with constant stirring until the temperature rises to 5 °C below the expected melting point. Then regulate the rate of increase to 1 °C per minute. The temperature at which the sample is observed to rise in the capillary tube is taken as the melting point.

Method 3
This method is applied to petrolatums.

(1) *Apparatus* Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line.

(2) *Procedure* Melt the sample slowly, with thorough stirring, until it reaches a temperature between 90 °C and 92 °C. Discontinue the heating, and allow the sample to cool to a temperature between 8 °C and 10 °C above the expected melting point. Chill the bulb of the thermometer to 5 °C, wipe, dry, and, while still cold, thrust into the molten sample to such a depth that approximately the lower half of the bulb is submerged. Withdraw it immediately, hold vertically, cool until the attached sample becomes dull, then dip for 5 minutes in water having a temperature not higher than 16 °C. Fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is 15 mm above the bottom of the test tube. Suspend the tube in water contained in a beaker at a temperature of about 16 °C, and raise the temperature of the bath to 30 °C at a rate of 2 °C per minute, then at a rate of 1 °C per minute until it reaches the melting point. Read the temperature at which the first drop leaves the thermometer. If the variations between each of three determinations are not more than 1 °C take the average of the three. If any of the variations is greater than 1 °C, make two additional determinations, and take the average of the five as the melting point.

32. Microbial Assay for Antibiotics

Microbial Assay for Antibiotics is a method to determine the antimicrobial potency of antibiotics based on their antimicrobial activities. Unless otherwise specified, perform a test according to the following methods. Perform an Assay in the individual monograph according to following test conditions of Cylinder-plate method (I), Standard curve method (II) or Turbidimetric method (III), and specifications in the individual monograph. Sterilize water, reagents, test solutions, measuring instruments and appliances to be used in the test.

I. Cylinder-plate method

1. Cylinder Use stainless steel cylinders with the following dimension: outside diameter 7.9 ~ 8.1 mm; inside diameter 5.9 ~ 6.1 mm; length 0.9 ~ 10.1 mm. The cylinders do not interfere with the test.

2. Culture media Unless otherwise specified, use media with following compositions. When 'peptone' is indicated as an ingredient of a medium, either meat peptone or casein peptone digested by pancreatin is applicable. Use 1 mol/L sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. In the case of the medium for Bacillus subtilis ATCC 6633, adjust the pH using ammonia TS, potassium hydroxide TS or 1 mol/L hydrochloric acid TS.

1) Agar media for seed and base layer

(1) Medium for *Bacillus subtilis* ATCC 6633

① Peptone	5.0 g
Meat extract	3.0 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

② Peptone	5.0 g
Meat extract	3.0 g
Sodium citrate	10.0 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Medium for *Micrococcus luteus* ATCC 9341

① Meat peptone	6.0 g
Pancreatin-digested casein	4.0 g
Meat extract	1.5 g
Glucose	1.0 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

② 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, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(3) Medium for *Staphylococcus aureus* ATCC 6538P

① Agar media for seed layer

Peptone	6.0 g
Pancreatin-digested 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, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

② Agar media for base layer

Peptone	6.0 g
Yeast extract	3.0 g
Meat extract	1.5 g

Glucose	10.0 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(4) Medium for *Saccharomyces cerevisiae* ATCC 9763

① Agar media for seed layer

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, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

(5) Medium for *Escherichiacoli* NIHJ

① Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(6) Medium for other organisms

① Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

2) Agar media for transferring test organisms

(1) Medium for *Saccharomyces cerevisiae* ATCC 9763

① Peptone	5.0 g
Meat extract	2.0 g
Glucose	15.0 g
Sodium dihydrogenphosphate	1.0 g
Magnesium sulfate	0.5 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

(2) Medium for other organisms

① Meat peptone	6.0 g
Pancreatin-digested 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, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

② Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	13.0 ~ 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

3) Liquid media for suspending test organisms

(1) Medium for *Saccharomyces cerevisiae* ATCC 9763

Peptone 10.0 g

Glucose 20.0 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 5.6 to 5.8 after sterilization.

(2) Medium for other organisms

Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization.

3. Test organisms Use the test organism specified in the individual monograph.

4. Preparation of suspension of test organism or test spores Prepare as specified in the individual monograph.

Unless otherwise specified, prepare suspensions of test organism cultures or test spores as follows, when one of *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, *Pseudomonas aeruginosa* NCTC 10490, *Saccharomyces cerevisiae* ATCC 9763, *Bacillus subtilis* ATCC 6633 or *Bacillus subtilis* ATCC 1768E is used as a test organism. Unless otherwise specified, prepare suspension of test organism or test spores by mixing 0.5 ~ 2.0mL of test organism suspension obtained through the procedure in 1), 2), 3), or 4) and 100mL of agar media previously melted and cooled down to 48 ℃.

1) Preparation of suspension of *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Escherichia coli* ATCC 9637, *Escherichia coli* ATCC 10536, or *Escherichia coli* NIHJ Inoculate the test organism onto the slant of the agar media for transferring test organisms in I 2 2) (2)①, incubate at 32 ~ 37 ℃ for 16 ~ 24 hours. The subculture is performed at least three times. Inoculate this sub-cultured test organism onto another slant made from about 9mL (test tube inner diameter : 16 mm) of the agar media for transferring test organisms in I 2 2) (2)①, incubate at 32 ~ 37 ℃ for 16 ~ 24 hours. Scrape away and suspend the resulting growth from the agar surface in 10mL of Isotonic Sodium Chloride Injection, and use this as a suspension of the test organism. Store the suspension at a temperature not exceeding 5 °C. Use the suspension of *Staphylococcus epidermidis* ATCC 12228 within 5 days, and use the suspension of the others within 7 days.

2) Preparation of suspension of *Pseudomonas aeruginosa* NCTC 10490 or *Micrococcus luteus* ATCC 9341 Inoculate the test organism onto the slant of the agar media for transferring test organisms in I 2 2) (2) ①, incubate at 25 ~ 26 °C for 40 ~ 48 hours in case of *Pseudomonas aeruginosa* NCTC 10490, and incubate at 25 ~ 26 °C for 24 ~ 48 hours or at 32 ~ 37 °C for 16 ~ 24 hours in case of *Micrococcus luteus* ATCC 9341. The subculture should be performed at least three times. Inoculate this subcultured test organism onto another slant made from about 9mL (test tube inner diameter: 16 mm) of the agar media for transferring test organisms in I 2 2) (2)①, incubate at 25 ~ 26 °C for 40 ~ 48 hours in case of *Pseudomonas aeruginosa* NCTC 10490, and incubate at 25 ~ 26 ℃ for 24 ~ 48 hours or at 32 ~ 37 °C for 16 ~ 24 hours in case of *Micrococcus luteus* ATCC 9341. Scrape away and suspend the resulting growth from the agar surface in 10mL of Isotonic Sodium Chloride Injection, and use this as a suspension of the test organism. Store the suspension at a temperature not exceeding 5 °C. Use the suspension within 2 days in case of *Pseudomonas aeruginosa*, and use the suspension of *Micrococcus luteus* within 5 days.

3) Preparation of suspension of *Saccharomyces cerevisiae* ATCC 9763 Inoculate the test organism onto the slant of the agar media for transferring test organisms in I 2 2) (1), incubate at 25 ~ 26 °C for 40 ~ 48 hours. The subculture is performed at least three times.

Inoculate this subcultured test organism onto another slant (test tube inner diameter: 16 mm) made from about 9mL of the agar media for transferring test organisms in I 2 2) (1), incubate at 25 ~ 26 °C for 40 ~ 48 hours. Scrape away and suspend the resulting growth from the agar surface in 10mL of Isotonic Sodium Chloride Injection, and use this as a suspension of the test organism. Store the suspension at a temperature not exceeding 5 °C. Use the suspension within 30 days.

4) Alternative method Incubate the test organism in 1), 2), or 3) on the slant of the agar media for transferring test organisms according to the above methods 1), 2), or 3). Scrape away and suspend the resulting growth from the agar surface in 3mL of Isotonic Sodium Chloride Injection. Inoculate this suspension of the test organism onto the surface of 300mL of the agar media for transferring test organisms in a Roux bottle, and spread evenly with glass spreader. Incubate at a specified temperature. Scrape away and suspend the resulting growth from the agar surface in an appropriate amount (usually about 50mL) of isotonic sodium chloride solution, and add Isotonic Sodium Chloride Injection to make the suspension of the test organism. Unless otherwise specified, dilute this suspension with Isotonic Sodium Chloride Injection about ten fold to make the suspension so that the transmission ratio is 25 % at 580 nm. In case of *Pseudomonas aeruginosa* NCTC 10490, use the suspension without dilution.

5) Preparation of suspension of *Bacillus subtilis* ATCC 6633 and *Bacillus subtilis* ATCC 1768E To the resulting growth of the test organism onto the slant of the agar media for transferring test organisms in I 2 2) (2)Ø1 , add about 3mL of Isotonic Sodium Chloride Injection and suspend. Inoculate this suspension of the test organism onto the Roux bottle slant surface of 300mL of the agar media for transferring test organisms in I 22) (2)Ø1 , and spread evenly with glass spreader. Incubate at 32 ~ 37 °C for not less than one week to prepare spores. Suspend the spores in 100mL of Isotonic Sodium Chloride Injection, heat at 65 °C for 30 minutes, and then centrifuge. Wash the spore sediment three times with about 50mL of Isotonic Sodium Chloride Injection by means of centrifugation. Resuspend the spore sediment in 100mL of sterile water or Isotonic Sodium Chloride Injection, and heat again at 65 ℃ for 30 minutes to prepare the spore suspension. Store the spore suspension at a temperature not exceeding 5 ℃, and use within 6 months. Determine the volume of spore suspension used for 100mL of agar media for seed layer by choosing a dilution to give a clear and definite zone of growth inhibition. Prepare agar media for seed layer by mixing the decided amount of spore suspension and 100mL of agar media previously melted and cooled down to 48 ℃. Unless otherwise specified, usually add 0.1 ~ 1.0mL of the spore suspension to 100mL of agar media for seed layer.

5. Preparation of cylinder agar plates Unless otherwise specified, dispense 20mL of the melted agar medium for the base layer into each Petri dish of 90 mm in inside diameter, and dispense 21mL of the melted agar medium for the base layer into Petri dish of 100 mm in inside diameter. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden. Use the plates within the day. Dispense 4.0mL of the seeded agar layer, which is specified in the individual monograph or 4 on an agar base layer plate in a Petri dish, and spread evenly over the surface before hardening, and allow it to harden at room 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 in case of about 90 mm inside diameter Petri dish and 28 mm in case of about 100 mm inside diameter Petri dish). In placing cylinder, drop a cylinder vertically from 10 ~ 13 mm height.

6. Standard solution Use both a standard solution of high concentration and one of low concentration, as specified in the individual monograph. A high concentration standard solution is symbolized as S_H and a low concentration standard solution is symbolized as S_L.

7. Test solution Use both a test solution of high concentration and one of low concentration, as specified in the individual monograph. A high concentration test solution is symbolized as U_H and a low concentration standard solution is symbolized as U_L. The concentration of test solution specified in the individual mono-

graph could be prepared within the concentration described ± 5 %.

8. Procedure Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. Apply S_H and SL to a pair of cylinders set opposite each other on each plate. Apply U_H and U_L to the remaining 2 cylinders. Incubate the plates at 32 ℃ to 37 ℃ for 16 to 20 hours. After incubation, measure the diameter (mm) of circular inhibition zones with a precision that can discriminate differences of at least 0.5 mm.

9. Estimation of potency The following correlation between the potency (P) of solution in a cylinder and the diameter (d) of zone of inhibition is established.

$$d = \alpha \times \log P + \beta \quad \text{(where, } \alpha \text{ and } \beta \text{ are constants)}$$

If necessary, ascertain the values in the above equation. Based on this equation, estimate the potency of the test solutions by application of the following equation.

$$\text{Amount (potency) of sample} = A \times \text{Potency of } S_H \text{ per mL} \times \text{Dilution factor of } U_H$$

$$\log A = \frac{I \times V}{W}$$

$$I = \log \frac{\text{potency of } S_H}{\text{potency of } S_L}$$

$$V = (\sum U_H + \sum U_L) - (\sum S_H + \sum S_L)$$

$$W = (\sum U_H + \sum S_H) - (\sum U_L + \sum S_L)$$

The sum of the diameter (mm) of the inhibitory zone measured in each plate for S_H, S_L, U_H and U_L is designated as ΣS_H, ΣS_L, ΣU_H, and ΣU_L, respectively.

II. Standard curve method Unless otherwise specified, comply with the requirements of Cylinder- plate method, such as cylinder, media, test organisms, preparation of suspensions of test organisms or spores. Also, comply with the specifications for media etc., in the individual monograph, if specified.

1. Preparation of cylinder-agar plates Prepare as directed in I 5. Use about 100 mm (inside diameter) Petri dishes, 6 cylinders are set on the circumference of a circle 28 mm, and the individual cylinders are equally spaced from one another with about 60° interval.

2. Standard solution Use the standard solution specified in the individual monograph.

3. Test solution Use the test solution specified in the individual monograph. The concentration of test solution specified in the individual monograph could be prepared within the concentration described ± 5 %.

4. Procedure Use the concentrations of standard solution and standard middle concentration solution specified in the individual monograph, and make a standard 1300 General Tests, Processes and Apparatus 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 standard middle concentration solution 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 test solution. Place 6 cylinders on each agar plate, apply standard middle concentration solution 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 circular inhibition zones with a precision that can discriminate differences of at least 0.5 mm.

5. Estimation of potency Average the diameters (d) of zone of inhibition from each concentration of the standard solution and standard middle concentration solution in every assay set, and average the diameters (d) of zone of inhibition from standard middle concentration solution in all assay set which is the average value for correction. This average value for correction is used for correction. When the average values of standard middle concentration solution 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 middle concentration solution is19.8 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 zone of inhibition from the test solution and standard middle concentration solution in an assay set. If the average value of the test solution is larger than the average value of the standard middle concentration solution, make a correction by adding the difference value to the diameter of 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 middle concentration solution, make a correction by subtracting the difference value from the diameter of zone of inhibition representing the center on the standard curve. Estimate the potency of test solution by extrapolating the diameter of zone of the test solution on the standard curve. Calculate the potency of test sample by multiplying the dilution factor. If the standard dilutions of five concentration 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 zone of inhibition for the lowest concentration of the standard curve

H: calculated value of the diameter of zone of inhibition for the highest concentration of the standard curve

c: the average value for correction

a: the value of corrected average value of diameter of zone of inhibition from the lowest concentration of the standard solution

b, d and e: the values of corrected average values of diameter of zone of inhibition from each sequentially increased concentration standard solution.

Ⅲ. Turbidimetric method

1. Test organisms Unless otherwise specified, use *Klebsiella pneumonia* ATCC 10031, *Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 10536 as a test organism.

2. Culture media 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.

1) Agar media for transferring test organisms

Peptone	6.0 g
Pancreatin-digested 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, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

2) Liquid media for suspending 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
Dipotassium hydrogen phosphate	3.68 g
Potassium dihydrogen phosphate	1.32 g

Weigh the above ingredients, dissolve in 1000mL of water, and adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization. Disodium hydrogen phosphate anhydrous (3.0 g) may be used instead of dipotassium hydrogen phosphate (3.68 g).

3. Preparation of suspensions of test organisms 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 slant of agar media for transferring test organisms, and incubate at 32 ~ 37 °C for 16 ~ 18 hours. Use the cultured organism to make a suspension of test organism as follows.

1) Preparation of suspension of *Klebsiella pneumonia* ATCC 10031 Suspend the test organism in about 5mL of sterile water, inoculate this suspension of the test organism onto the surface of 300mL of the agar media for transferring test organisms in a Roux bottle, and spread evenly with glass spreader. Incubate at 32~37 ℃ for 16 ~ 24 hours. Scrape away and suspend the resulting growth from the agar surface in an appropriate amount (usually about 50mL) 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 6.0mL of this suspension to 100mL of the liquid media for suspending test organism spreviously chilled to about 15 ℃, and use the resulting suspension as the suspension of test organism.

2) Preparation of suspension of *Staphylococcus aureus* ATCC 6538P Suspend the test organism in about 10mL of the liquid media for suspending test organisms. To this suspension add about 15mL of the liquid media for suspending test organisms to make the suspension so that the transmission ratio is 85 % at 650 nm. Before use, add 4.0mL of this suspension to 100mL of the liquid media for suspending test organisms, previously chilled to about 15 °C, and use the resulting suspension as the suspension of test organism.

3) Preparation of suspension of *Escherichia coli* ATCC 10536 Suspend the test organism in an appropriate amount of sterile water to make the suspension so that the transmission ratio is 90 % at 650 nm. Store the suspension at a temperature not exceeding 5 °C, and use within 14 days. Before use, add 2.0mL of this suspension to 100mL of the liquid media for suspending test organisms, previously chilled to about 15 °C, and use the resulting suspension as the suspension of test organism.

4. Standard solution Use the standard solutions specified in the individual monograph.

5. Test solution Use the test solution specified in the individual monograph. The concentration of test solution specified in the individual monograph could be prepared within the concentration described ± 5 %.

6. Procedure Unless otherwise specified, proceed as follows: Distribute 1.0mL of each concentration of the standard solution, the test solution, and water used as a control, into each set composed of 3 test tubes (about 13.5 ~ 14.5 mm in inside diameter and about 130 mm in length). Add 9.0mL of the suspension of the test organism to each tube, and then incubate in a water bath maintained at 35 ~ 37 °C for 3 ~ 4 hours. After incubation, add 0.5mL of formaldehyde solution(1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

7. Estimation of potency Average the transmittance or absorbance values of each concentration of the standard solution, the test solution and water used as a control, 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.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

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.

33. Microbial Limit Test

The Microbial limit test includes tests for total viable count and specified microbial species. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If samples are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

I. Microbiological Examination of Non-sterile Products: Total viable aerobic count

1) Introduction

This test determines the mesophilic bacteria and fungi which grow under aerobic conditions. The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

The methods are not applicable to products containing viable microorganisms as active ingredients. Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopial method has been demonstrated.

2) General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any microorganisms which are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose their efficacy and their absence of toxicity for microorganisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with inactivators used must be demonstrated.

3) Total viable count Methods

Use the membrane filtration method, or the plate-count methods, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of microorganisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

4) Growth promotion Test, Suitability of the Counting Method and Negative Controls

i) ***General requirement*** The ability of the test to detect microorganisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

ii) ***Preparation of test strains*** Use standardised stable suspensions of test strains or prepare as stated below.

Seed lot culture maintenance techniques (seed-lot systems) are use so that the viable microorganisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table I-1.

Table I-1 Preparation and use of test microorganisms

Microorganism	Preparation of test strain	Growth promotion		Suitability of counting method in the presence of the product	
		Total aerobic microbial count	Total yeasts and moulds count	Total aerobic microbial count	Total yeasts and moulds count

Staphylococcus aureus Such as ATCC 6538, NCIMB 9518, CIP 4.83, NBRC 13276 or KCTC 3881	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 - 35 °C 18 – 24h	Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days	
Pseudomonas aeruginosa Such as ATCC 9027, NCIMB 8626 CIP 82.118, NBRC 13275 or KCTC 2513	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 - 35 °C 18 – 24h	Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days	
Bacillus subtilis Such as ATCC 6633, NCIMB 8054, CIP 52.62, NBRC 3134 or KCTC 1021	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 - 35 °C 18 – 24h	Soybean-Casein Digest Agar Medium and Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days		Soybean-Casein Digest Agar Medium/MPN Fluid Soybean-Casein Digest Medium ≤ 100 CFU 30 - 35 °C ≤ 3 days	
Candida albicans Such as ATCC 10231, NCPF 3179, IP 48.72, NBRC 1594 or KCTC 7965	Sabouraud Glucose Agar Medium or Fluid Sabouraud Glucose Medium 20 - 25 °C 2 - 3 days	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 - 35 °C ≤ 5 days	Sabouraud Glucose Agar Medium ≤ 100 CFU 20 - 25 °C ≤ 5 days	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 - 35 °C ≤ 5 days MPN: not applicable	Sabouraud Glucose Agar Medium ≤ 100 CFU 20 - 25 °C ≤ 5 days
Aspergillus brasiliensis Such as ATCC 16404, IMI 49007, IP 1431.83, NBRC 9455, KCTC 6317 or KCTC 6196	Sabouraud Glucose Agar Medium or Potato Dextrose Agar Medium 20 - 25 °C 5 – 7 days, or until good sporulation is achieved	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 - 35 °C ≤ 5 days	Sabouraud Glucose Agar Medium ≤ 100 CFU 20 - 25 °C ≤ 5 days	Soybean-Casein Digest Agar Medium ≤ 100 CFU 30 - 35 °C ≤ 5 days MPN: not applicable	Sabouraud Glucose Agar Medium ≤ 100 CFU 20 - 25 °C ≤ 5 days

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0) or Phosphate Buffer (pH 7.2) to make test suspensions; to suspend *Aspergillus brasiliensis* spores, 0.05 % of polysorbate 80 may be added to the buffer. Use the suspensions within 2h or within 24h if stored at 2–8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2–8 °C for a validated period of time.

iii) *Negative control*

To verify testing conditions, a negative control is performed 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 under 5. Testing of Products. A failed negative control requires an investigation.

iv) *Growth promotion of media* Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/ plates of Fluid Soybean-Casein Digest Medium and Soybean-Casein Digest Agar Medium with a small number (not more than 100 CFU) of the microorganisms indicated in Table I-1, using a separate portion/ plate of medium for each. Inoculate plates of Sabouraud Glucose Agar Medium with a small number (not more than 100 CFU) of the micro-

organisms indicated in Table I-1, using a separate plate of medium for each. Incubate in the conditions described in Table I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculums. For a freshly prepared inoculum, growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Liquid media are suitable if clearly visible growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

v) *Suitability of the counting method in the presence of product*

① **Preparation of the sample** The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

(i) *Water-soluble products* Dissolve or dilute (usually a1in 10 dilution is prepared) the product to be examined in Buffered Sodium Chloride- Peptone Solution(pH 7.0), Phosphate Buffer (pH 7.2) or Fluid Soybean-Casein Digest Medium. If necessary adjust top H6–8. Further dilutions, where necessary, are prepared with the same diluents.

(ii) *Non-fatty products in soluble in water*: Suspend the product to be examined (usually a1in 10 dilution is prepared) in Buffered Sodium Chloride- Peptone Solution (pH 7.0), Phosphate Buffer (pH 7.2) or Fluid Soybean-Casein Digest Medium. A surface- active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wetable substances. If necessary adjust to pH 6–8. Further dilutions, where necessary, are prepared with the same diluents.

(iii) *Fatty products* Dissolve in isopropyl myristate, sterilized by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent, heated if necessary to not more than 40 °C, of in exceptional cases to not more than 45 °C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the prewarmed chosen diluent to make a1in 10 dilution of the original product. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial ten fold dilutions may be prepared using the chosen diluents containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

(iv) *Fluids or solids in aerosol form* A septically transfer the product in to a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

(v) *Transdermal patches* Remove the protective cover sheets (releaseliner) of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluents containing in activators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 min.

② **Inoculation and dilution** Add to the sample prepared as described above ① and to a control (with no test material included) a sufficient volume of the 100 CFU. The volume of the suspension of the inoculum should not exceed 1 % of the volume of diluted product. To demonstrate acceptable microbial recovery from the product, the lowest possible factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed.

If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

③ **Neutralization / removal of antimicrobial activity** The number of microorganisms recovered from the prepared sample diluted as described in ② and incubated following the procedure described in ④, is compared to the number of microorganisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluents, (3) membrane filtration or (4) a combination of the above measure.

Neutralizing agents ― Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table I-2). They may be added to the chosen diluents or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for microorganisms must be demonstrated by carrying out a blank with neutralizing agents, without product.

Table I-2 Common neutralizing agents/ method for interfering substances

Interfering substance	Potential neutralizing agents/ method
Glutaraldehyde, Mercurials	Sodium hydrogen sulfite (Sodium bisulfate)
Phenolics, Alcohol, Aldehydes, Sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), Parahydroxybenzoates (Parabens), Bis-biguanides	Lecithin
QAC, Parabens, Iodine	Polysorbate
Mercurials	Thioglycollate

Mercurials, Halogens, Aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the microorganism. However, it is possible that the product only inhibits some of the microorganisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

④ **Recovery of micro- organism in the presence of product** For each of the microorganisms listed in Table I-1, separate tests are performed. Only microorganisms of the added test strain are counted.

(i) Membrane filtration method Use membrane filters having a nominal pore size not greater than 0.45 μm. The type of filter material is chosen in such away that the bacteria- retaining efficiency is not affected by the components of the sample to be investigated. For each of the microorganisms listed in TableI-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under ① to 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 Medium. For the determination of total combined yeasts/ moulds count (TYMC) transfer the membrane to the surface of Sabouraud Glucose Agar medium. Incubate the plates as indicated in Table I-1. Perform the counting

(ii) Plate-count methods Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

(a) Pour-plate method For Petri dishes 9 cm in diameter, add to the dish 1mL of the sample prepared as described under ① to ③ and 15 –20mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium, both media being at not more than 45 °C. If larger Petri dishes are used, the amount agar medium is increased accordingly. For each of the microorganisms listed in TableI-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(b) Surface-spread method For Petri dishes 9 cm in diameter, add 15 – 20mL of Soybean-Casein Digest Agar Medium or Sabouraud Glucose Agar Medium at about 45 °C to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabine torin an incubator. For each of the micro- organisms listed in Table Ⅰ-1 , at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1mL of the sample prepared as described under ① to ② over the surface of the medium. Incubate and count as prescribed under (ii)(a).

(iii) Most-probable-number (MPN) method The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows. Prepare a series of at least 3 serial tenfold dilutions of the product as described under ① to ③. From each level of dilution, 3 aliquots of 1 g or 1mL are used to inoculate 3 tubes with 9 – 10mL of Fluid Soybean-Casein Digest Medium. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be add to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at 30 – 35 °C for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or Soybean-Casein Digest Agar Medium, for 1 – 2 days at the same temperature and use these results. Determine the most probable number of microorganisms per gram or milliliter of the product to be examined from Table I-3.

Table I-3 Most-probable-number values of microorganisms

Observed combinations of numbers of tubes Showing growth in each set			**MPN per g or permL of product**	**95 per cent Confidence limits**
Number of g ormL of product per tube				
0.1	**0.01**	**0.001**		
0	0	0	< 3	0 – 9.4
0	0	1	3	0.1 - 9.5
0	1	0	3	0.1 – 10
0	1	1	6.1	1.2 - 17
0	2	0	6.2	1.2 - 17
0	3	0	9.4	3.5 – 35
1	0	0	3.6	0.2 – 17
1	0	1	7.2	1.2 – 17
1	0	2	11	4 – 35
1	1	0	7.4	1.3 – 20
1	1	1	11	4 - 35
1	2	0	11	4 - 35

1	2	1	15	5 - 38
1	3	0	16	5 – 38
2	0	0	9.2	1.5 – 35
2	0	1	14	4 - 35
2	0	2	20	5 - 38
2	1	0	15	4 - 38
2	1	1	20	5 - 38
2	1	2	27	9 - 94
2	2	0	21	5 – 40
2	2	1	28	9 - 94
2	2	2	35	9 – 94
2	3	0	29	9 - 94
2	3	1	36	9 - 94
3	0	0	23	5 - 94
3	0	1	38	9 - 104
3	0	2	64	16 – 181
3	1	0	43	9 – 181
3	1	1	75	17 – 199
3	1	2	120	30 – 360
3	1	3	160	30 – 380
3	2	0	93	18 – 360
3	2	1	150	30 – 380
3	2	2	210	30 – 400
3	2	3	290	90 – 990
3	3	0	240	40 – 990
3	3	1	460	90 – 1980
3	3	2	1100	200 - 4000
3	3	3	> 1100	

vi) Results and interpretation When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in ② in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inocula must be within 95 per cent confidence limits of the results obtained with the control. If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

5) Testing of Products

i) ***Amount used for the test*** Unless otherwise prescribed, use 10 g or 10mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches. The amount to be tested may be reduced for Active Pharmaceutical Ingredients that will be formulated in the following conditions: the amount per dosage unit (e.g., tablets, capsules, injections) is less than or equal to 1 mg or the amount per gram or millilitre (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10mL of the product. For materials used as Active Pharmaceutical Ingredients where sample quantity is limited or batch size is extremely small (i.e., less than 1000mL or 1000 g), the amount tested shall be 1 % of the batch unless a lesser amount is prescribed or justified and authorized. For products where the total number of entities in a batch is less than 200 (e. g. samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100. Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

ii) ***Examination of the product*** **① Membrane filtration method** Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in section 4) and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable. For the determination of TAMC, transfer one of the membrane filters to the surface of Soybean-Casein Digest Agar Medium. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud Glucose Agar Medium. Incubate the plate of Soybean-Casein Digest Agar Medium at 30 – 35 °C for 3 – 5 days and the plate of Sabouraud Glucose Agar Medium at 20 – 25 °C for 5 – 7 days. Calculate number of CFU per gram or per millilitre of product. When examining transdermal patches, filter 10 % of the volume of the preparation described under 4) iv)① separately through each of 2 sterile filter membranes. Transfer one membrane to Soybean-Casein Digest Agar Medium for TAMC and the other membrane to Sabouraud Glucose Agar Medium for TYMC.

② *Plate-count methods* (i) Pour-plate method Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of Soybean-Casein Digest Agar Medium at 30 – 35 °C for 3 – 5 days and the plates of Sabouraud Glucose Agar Medium at 20 – 25 °C for 5 – 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per milliliter of product.

(ii) ***Surface-spread method*** Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

③ **Most-probable-number method** Prepare and dilute the sample using a method that has been shown to be suitable as described in section 4). Incubate all tubes for 3 – 5 days at 30 – 35 °C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of microorganisms per gram or millilitre of the product to be examined from Table I-3.

iii) ***Interpretation of the results*** The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using Soybean-Casein Digest Agar Medium; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using Sabouraud Glucose Agar Medium; 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 Glucose Agar Medium containing antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC. When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

101 CFU : maximum acceptable count = 20,

102 CFU : maximum acceptable count = 200,

103 CFU : maximum acceptable count = 2000, and so forth.

The recommended solutions and media are described in Tests for specified microorganisms.

II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms

1) Introduction These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The tests described hereafter will allow determination of the absence of, or limited occurrence of specified microorganisms which may be detected under the conditions described. The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of sample to be taken and interpret the results as stated below. Alternative microbiological procedures, including automated methods may be sued, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

2) General Procedures The preparation of samples is carried out as described in I. Total viable aerobic count. If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in I. Total viable aerobic count. If surface-active substances are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with inactivators used must be demonstrated as described in I. Total viable aerobic count.

3) Growth promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls The ability of the test to detect microorganisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

i) Preparation of test strains Use standardised stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

① **Aerobic microorganisms** Grow each of the bacterial test strains separately in containers containing Fluid Soybean-Casein Digest Medium or on Soybean-Casein Digest Agar Medium at 30 - 35 °C for 18 – 24 hours. Grow the test strain for Candida albicans separately on Sabouraud Glucose Agar Medium or in Fluid Sabouraud Glucose Medium at 20 - 25 °C for 2 - 3 days.

Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83, NBRC 13276 or KCTC 1927 *Pseudomonas aeruginosa* such as ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275 or KCTC 2513 Escherichia coli such as ATCC 8739, NCIMB 8545, CIP 53.126, NBRC 3972 or KCTC 2571 Salmonella enterica subsp. enterica serovar Typhimurium such as ATCC 14028 or, as an alternative, Salmonella enterica subsp. enterica serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39, Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72, NBRC 1594 or KCTC 7965.

Use Buffered Sodium Chloride-Peptone Solution (pH 7.0) or Phosphate Buffer (pH 7.2) to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 – 8 °C

② **Clostridia** Use Clostridium sporogenes such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under an aerobic conditions in Reinforced Clostridial Medium at 30 – 35 °C for 24 – 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of Cl. sporogenes, as table spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8 °C for a validated period.

ii) Negative control To verify testing conditions a negative control is performed 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 under 3. A failed negative control required an investigation.

iii) Growth promotion and inhibitory properties of the media Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Verify suitable properties of relevant media as described in Table II-1.

Table II-1 Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
Test for bile-tolerant gram-negative bacteria		
Fluid Enterobacteria Enrichment Broth Mossel Medium	Growth promoting	*E. coli* *P. aeruginosa*
	Inhibitory	*S. aureus*
VRB (Violet/Red/Bile) Agar with glucose	Growth promoting + Indicative	*E. coli* *P. aeruginosa*
Test for *Escherichiacoli*		
Fluid MacConkey Broth Medium	Growth promoting	*E. coli*
	Inhibitory	*S. aureus*
MacConkey Agar Medium	Growth promoting + Indicative	*E. coli*
Test for Salmonella		
Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium	Growth promoting	*Salmonella enterica* subsp.*enterica*serovarTyphimurium or *Salmonella enterica* subsp.*enterica*serovarAbony
	Inhibitory	*S. aureus*
XLD (Xylose-Lysine-Desoxycholate) Agar Medium	Growth promoting + Indicative	*Salmonella enterica* subsp.*enterica*serovarTyphimurium or *Salmonella enterica* subsp.*enterica*serovarAbony
	Indicative	*E. coli*
Test for *Pseudomonasaeruginosa*		
Cetrimide Agar Medium	Growth promoting	*P. aeruginosa*
	Inhibitory	*E. coli*
Test for *Staphylococcusaureus*		
Mannitol Salt Agar Medium	Growth promoting + Indicative	*S. aureus*
	Inhibitory	*E. coli*
Test for Clostridia		
Reinforced Clostridial Medium	Growth promoting	*Cl. sporogenes*
Columbia Agar Medium	Growth promoting	*Cl. sporogenes*
Test for *Candidaalbicans*		
Fluid Sabouraud Glucose Medium	Growth promoting	*C. albicans*
Sabouraud Glucose Agar Medium	Growth promoting + Indicative	*C. albicans*

Test for growth promoting properties, liquid media Inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the microorganism comparable to that previously obtained

with a previously tested and approved batch of medium occurs.

Test for growth promoting properties, solid media Perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for inhibitory properties, liquid or solid media Inoculate the appropriate medium with at least 100 CFU of the appropriate microorganism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the microorganism occurs.

Test for indicative properties Perform surface spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

iv) Suitability of the test method For each product to be tested perform sample preparation as described in the relevant paragraph in section 4). Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of microorganisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 4) using the shortest incubation period prescribed. The specified microorganisms must be detected with the indication reactions as described in section 4). Any antimicrobial activity of the product necessitates a modification of the test procedure [see 4) iv) ③ of Total viable aerobic count]. If for a given product the antimicrobial activity with respect to a microorganism for which testing is prescribed cannot be neutralised, then it is to be assumed that the inhibited microorganism will not be present in the product.

4) TestingofProducts

i) Bile-tolerant gram-negative bacteria

① **Sample preparation and pre-incubation** Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Ⅰ. Total viable aerobic count, but using Fluid Soybean-Casein Digest Medium 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 but not more than 5 hours).

② **Test for absence** Unless otherwise prescribed use the volume corresponding to 1 g of the product, as prepared in 3.1.1. to inoculate Fluid Enterobacteria Enrichment Broth Mossel Medium. Incubate at 30 - 35 °C for 24 – 48 hours. Subculture on plates of VRB(Violet/Red/Bile) Agar with glucose. Incubate at 30 – 35 °C for 18 – 24 hours. The product complies with the test if there is no growth of colonies.

③ **Quantitative test**

(i) Selection and subculture Inoculate suitable quantities of Fluid Enterobacteria Enrichment Broth Mossel Medium with the preparation as described under ① and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1mL, 0.01mL and 0.001mL) of the product to be examined. Incubate at 30 – 35 °C for 24 – 48 hours. Subculture each of the cultures on a plate of VRB(Violet/Red/Bile) Agar with glucose. Incubate at 30 - 35 °C for 18 – 24 hours.

(ii) Interpretation Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity of the product that gives a negative result. Determine from Table II-2 the probable number of bacteria.

Table II-2 Interpretation of results

Results for each quantity of product			Probable number of bacteriaper gram ormL of product
0.1 g or 0.1mL	**0.01 g or 0.01mL**	**0.001 g or 0.001mL**	
+	+	+	more than 10^3
+	+	-	less than $10^3$and more than10^2
+	-	-	less than $10^2$and more than10
-	-	-	less than 10

ii) Escherichia coli

① **Sample preparation and pre-incubation** Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Ⅰ. Microbial Enumeration Tests and use 10mL or the quantity corresponding to 1 g or 1mL to inoculate a suitable amount (determined as described under 3) iv)) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35 °C for 18 – 24 hours.

② **Selection and subculture** Shake the container, transfer 1mL of Fluid Soybean-Casein Digest medium to 100mL of Fluid MacConky Broth Meedium and incubate at 42 - 44 °C for 24 – 48 hours. Subculture on a plate of MacConkey Agar Medium at 30 – 35 °C for 18 – 72 hours.

③ **Interpretation** Growth of colonies indicates the possible presence of E.coli. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

***iii)* Salmonella**

① **Sample preparation and pre-incubation** Prepare the product to be examined as described in Ⅰ. Total viable aerobic count and use the quantity corresponding to not less than 10 g or 10mL to inoculate a suitable amount (determined as described under 3) iv)) of Fluid

Soybean-Casein Digest Medium, mix and incubate at 30 - 35 °C for 18 – 24 hours.

② **Selection and subculture** Transfer 0.1mL of Fluid Soybean-Casein Digest Medium to 10mL of Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium and incubate at 30 - 35 °C for 18 – 24 hours. Subculture on plates of XLD (Xylose-Lysine-Desoxycholate) Agar Medium. Incubate at 30 – 35 °C for 18 – 48 hours.

③ **Interpretation** The possible presence of Salmonella is indicated by the growth of well-developed, red colonies, with or without black center. This i s confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

iv) *Pseudomonas aeruginosa*

① **Sample preparation and pre-incubation** Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Ⅰ. Total viable aerobic count and use 10mL or the quantity corresponding to 1 g or 1mL to inoculate a suitable amount (determined as described under 3) iv).) of Fluid Soybean-Casein Digest Medium and mix. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. Total viable aerobic count (4) v) ①.) through a sterile filter membrane and place in 100mL of Fliuid Soybean-Casein Digest Medium. Incubate at 30 – 35 °C for 18 – 24 hours.

② **Selection and subculture** Subculture on a plate of Cetrimide Agar Medium and incubate at 30 – 35 °C for 18 – 72 hours.

③ **Interpretation** Growth of colonies of colonies indicates the possible presence of P.aeruginosa. This is confirmed by identification tests.

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

v) *Staphylococcus aureus*

① **Sample preparation and pre-incubation** Prepare a sample using a 1 in 10 dilution of nor less than 1 g of the product to be examined as described in I. Microbial Enumeration Tests and use 10mL or the quantity corresponding to 1 g or 1mL to inoculate a suitable amount (determined as described under 3) iv).) of Fluid Soybean-Casein Digest Medium and homogenize. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. Total viable aerobic count (4) iv)①.) through a sterile filter membrane and place in 100mL of Fluid Soybean-Casein Digest Medium. Incubate at 30 – 35 °C for 18 – 24 hours.

② **Selection and subculture** Subculture on a plate if Mannitol Salt Agar Medium and incubate at 30 - 35 °C for 18 – 72 hours.

③ **Interpretation** The possible presence of S.aureus is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

vi) *Clostridia*

① **Sample preparation and heat treatment** Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined as described in I. Total viable aerobic 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.

② **Selection and subculture** Use 10mL or the quantity corresponding to 1 g or 1mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under 3) iv).) of Reinforced Clostridial Medium. Incubate under anaerobic conditions at 30 – 35 °C for 48 hours. After incubation, make subcultures from each tube on Columbia Agar Medium and incubate under anaerobic conditions at 30 – 35 °C for 48 – 72 hours.

③ **Interpretation** The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of Clostridia. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

vii) *Candida albicans*

① **Sample preparation and pre-incubation** Prepare the product to be examined as described in I. Microbial Enumeration Tests and use 10mL or the quantity corresponding to not less than 1 g or 1mL to inoculate 100mL of Fluid Sabouraud Glucose Medium and mix. Incubate at 30 - 35 °C for 3 – 5 days.

② **Selection and subculture** Subculture on a plate of Sabouraud Glucose Agar Medium and incubate at 30 – 35 °C for 24 – 48 hours.

③ **Interpretation** Growth of white colonies may indicate the presence of C.albicans. This is confirmed by identification tests. The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

The following section is given for information.

5) Recommended Solutions and Culture Media

The following solutions and culture media have been found satisfactory for the purpose for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used provided that their suitability can be demonstrated.

Stock buffer solution Transfer 34 g of potassium dihydrogen phosphate to a 1000mL volumetric flask, dissolve in 500mL of purified water, adjust to pH 7.1 to 7.3 with sodium hydroxide, add purified water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 – 8 °C.

Phosphate Buffer (pH 7.2) Prepare a mixture of water and stock buffer solution (800 : 1 V/V) and sterilize.

Buffered Sodium Chloride- Peptone Solution (pH 7.0)

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate (equivalent to 0.067 mol phosphate)	7.2 g
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave using a validated cycle.

Fluid Soybean-Casein Digest Medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25 °C. Sterilize in an autoclave using a validated cycle.

Soybean-Casein Digest Agar Medium

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 so that after sterilization it is 7.1 – 7.5 at 25 °C. Sterilize in an autoclave using a validated cycle.

Sabouraud Glucose Agar Medium

Glucose	40.0 g
Peptones (animal tissue and casein 1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25 °C. Sterilize in an autoclave using a validated cycle.

Potato Dextrose Agar Medium

Infusion from potatoes	200 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25 °C. Sterilize in an autoclave using a validated cycle.

Fluid Sabouraud Glucose Medium

Glucose	20.0 g
Peptone (animal tissue and casein 1:1)	10.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25 °C. Sterilize in an autoclave using a validated cycle.

Fluid Enterobacteria Enrichment Broth Mossel Medium

Gelatin peptone	10.0 g
Glucose	5.0 g
Bile salts	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 so that after sterilization it is 7.1 – 7.4 at 25 °C. Heat at 100 °C for 30 min and cool immediately

VRB (Violet/Red/Bile) Agar with glucose

Yeast extract	3.0 g
Gelatin peptone	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Water	1000 mL

Adjust the pH so that after sterilization it is 7.2 – 7.6 at 25 °C. Heat to boiling; do not heat in an autoclave.

Fluid MacConkey Broth Medium

Gelatin peptone	20.0 g
Lactose monohydrate	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Water	1000mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25 °C. Sterilize in an autoclave using a validated cycle.

MacConkey Agar Medium

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
Crystal violet	1 mg
Water	1000 mL

Adjust the pH so that after sterilization it is 6.9 – 7.3 at 25 °C. Boil for 1 min with constant shaking then sterilize in an autoclave using a validated cycle.

Fluid Rappaport Vassiliadis Salmonella Enrichment Medium

Soya peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dipotassium hydrogen phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	36 mg
Water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115 °C. The pH is to be 5.0 – 5.4 at 25 °C after heating and autoclaving.

XLD(Xylose-Lysine-Desoxycholate) Agar Medium

Xylose	3.5 g
L-Lysine	5.0 g
Lactose monohydrate	7.5 g
Sucrose	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	6.8 g
Ammonium iron (III) citrate	0.8 g
Water	1000 mL

Adjust the pH so that after heating it is 7.2 – 7.6 at 25 °C. Heat to boiling, cool to 50 °C and pour into Petri dishes. Do not heat in an autoclave.

Cetrimide Agar Medium

Gelatin peptone	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Water	1000 mL
Glycerol	10.0 mL

Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilization it is 7.0 – 7.4 at 25 °C. Sterilize in an autoclave using a validated cycle.

Mannitol Salt Agar Medium

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Beef 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 with shaking. Adjust the pH so that after sterilization it is 7.2 – 7.6 at 25 °C. Sterilize in an autoclave using a validated cycle.

Reinforced Clostridial Medium

Beef 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 with continuous stirring. If necessary, adjust the pH so that after sterilization it is about 6.6 – 7.0 at 25 °C. Sterilize in an autoclave using a validated cycle.

Columbia Agar Medium

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 so that after sterilization it is 7.1 – 7.5 at 25 °C. Sterilize in an autoclave using a validated cycle. Allow to cool to 45 – 50 °C; add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

34. Mineral Oil Test

The Mineral Oil Test is a method to test mineral oil in non-aqueous solvents for injections and for eye drops.

Procedure

Pour 10 mL of the sample into a 100mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol. Put a short-stemmed, small funnel on the neck of the flask, placing the stem of the funnel downward, and heat on a water–bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

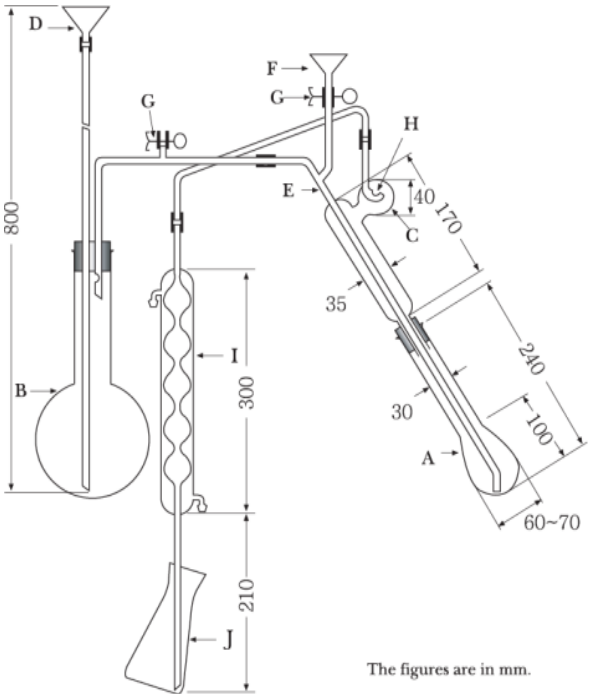
35. Nitrogen Determination (Semimicro-Kjeldahl Method)

The Nitrogen Determination is a method to determine ammonia in an organic substance in which the nitrogen is converted into ammonia nitrogen by thermal decomposition of the organic substance with sulfuric acid, and the ammonia liberated by alkali and trapped by distillation with steam is determined by titration.

Apparatus

Use the apparatus illustrated in the figure. It is thoroughly made of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Alternatively, apparatus can be used in which some of the procedures, such as digestion of organic substances, distillation of the liberated ammonia, and endpoint detection methods in titrimetry (e.g., potentiometric titration or titration by colorimeter) are automated.



A: Kjeldahl flask
B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and boiling tips for preventing bumping are added
C: Spray trap
D: Water supply tunnel
E: Steam tube
F: Funnel for addition of alkali solution to flask A
G: Rubber tubing with a clamp
H: A small hole having a diameter approximately equal to that of the delivery tube
I: Condenser, the lower end of which is beveled
J: Absorption flask

System suitability

If an automated apparatus is used, it is necessary to confirm periodically the suitability of the apparatus according to the following method.

Weigh accurately about 1.7 g of amidosulfuric acid (standard reagent), previously dried in a desiccators (in vacuum, silica gel) for about 48 hours, dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, and transfer to a digestion flask. When the test is performed as directed in the instrumental manual the nitrogen content (%) in amidosulfuric acid should ne determined between 14.2 % and 14.6 %.

Reagents, The Solution

Decomposition accelerator: Unless otherwise specified, use 1 g or a powdered mixture of 10 g of potassium sulfate and 1 g of cupper (II) sulfate pentahydrate. The composition and amount of the digestion accelerator may be modified if it is confirmed that the modified one give almost the same results using the sample as those obtained from the conventional catalyst.

Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet accurately a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N: mol. wt. 14.01), and place in the Kjeldahl flask A. Add the decomposition accelerator and wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask. Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide solution(30) drop by drop along the inside wall of the flask. Heat the flask gently unti lsulfuric acid condenses again at the neck of the flask. Stop heating when the solution has a clear blue color, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide solution(30) after cooling, and heat again. After cooling, add cautiously 20mL of water, cool the solution, and connect the flask to the distillation apparatus washed before hand by passing steam through it. To the absorption flask J, add 15mL of boric acid solution(1 in 25), 3 drops of bromocresol green-methyl red TS and a proper amount of water and the lower end of the condenser tube I is immersed into this solution. Add 30 mL of sodium hydroxide solution(2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, immediately close the clamp attached to the rubber tubing G, then begin the distillation with steam, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube I, rinsing the end part with a small quantity of water, and titrate the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.14007 mg of N

36. Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is based on the phenomenon that specific radio frequency radiation is absorbed by magnetic nuclei in a sample placed in a magnetic field.

Target nuclei are ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P, etc.

The spin quantum numbers, I, are values of 0, 1/2, 1, 3/2 ,…,n/2 (where n is integer), and I = 1/2 for 1H and 13C. When the magnetic nuclei are placed in a magnetic field, they are oriented in 2I+1 possible orientations (two energy levels for ^{1}H and ^{13}C). The transition between two successive quantized energy levels corresponding to the adjacent orientations can be induced by electromagnetic radiation with a suitable frequency, ν given by the following equation:

Where H_0 is the strength of the applied external magnetic field and γ is the gyromagnetic ratio, a constant characterizing a particular isotope.

$$= \frac{H_0}{2\,\mathrm{p}}$$

γ : the gyromagnetic ratio
H_0 : the strength of the applied external magnetic field

The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since this absorption coefficient (the transition probability) does not depend on the environments where the nuclei are located, the intensity of an absorption line is proportional to the number of nuclei (^{1}H) involving in the absorption. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state (relaxation) at various rates determined by characteristic time constants known as relaxation time. When a molecule is placed in the magnetic field, electrons of the molecule shield nuclei from the applied magnetic field. Therefore, nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift (δ), which is given by

$$= \frac{n_S - n_R}{n_R} + \quad _R$$

ν_S : Resonance frequency of the observed signal,
ν_R : Resonance frequency of the reference signal, and
δ_R: Chemical shift of the reference signal (in the case of the value not being 0)

The chemical shifts are normally expressed in ppm, a dimnensionless unit, by assuming the chemical shift of the reference compound as 0 ppm. When the chemical shift of the reference compound is not assumed to be 0 ppm, chemical shifts of samples are corrected accordingly.

ln addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei (nucleus having spin is a magnet by itself) by coupling through chemical bonds, resulting in an additional splitting of the signal. The spacing between two adiacent components of the signal is known as the spin-spin coupling constant J. Coupling constants are measured in hertz and are independent of the strength of the external magnetic field. The increased number of interacting nuclei will make multiplet pattern more complex.

From the NMR spectrum the following 4 parameters can be obtained: chemical shift, spin-spin coupling constant, resonance intensity (intensities of 1H are proportional to the number of nuclei and those of ^{13}C and others are susceptible to the nuclear Overhauser effect (NOE) and relaxation) and relaxation time. These parameters are useful for structural determination, identification and quantitative analysis of molecules. Spin decoupling, NOE, and two-dimensional NMR techniques are also available for structural analysis.

Spectrometer

Spectra are acquired using continuous wave NMR (CW-NMR) spectrometers, or pulse Fourier Transform NMR (FT-NMR) spectrometers.

Measurement

Prior to measurements, the sensitivity and resolution of the instrument is adjusted to the best conditions using the standard sample (ethylbenzene, o-dichlorobenzene or acetaldehyde) dissolved in an appropriate NMR solvent.

(i) The sample dissolved in a relevant solvent is transferred into an NMR tube. The reference compound can be added directly to the test solution (internal reference), or a sealed capillary tube containing the reference compound can be inserted into the NMR tube (external reference). The test solutions is made to be completely homogeneous. Various deuterated NMR solvents are used. The following considerations should be paid for selecting an appropriate solvent: (i) the solvent signals do not overlap with the sample signals, (ii) the sample is soluble in the solvent selected, and (iii) the solvent does not react with the sample. Furthermore, it is noted that chemical shifts can depend upon solvents employed, sample concentrations, and deuterium ion concentrations, and that viscous solutions usually give rather broad, poorly resolved spectra.

(ii) For the reference standards use the reagents for nuclear magnetic resonance spectroscopy. For 1H and ^{13}C spectra, tetramethylsilane(TMS) is usually used as the reference compound for samples dissolved in oryanic solvents. For samples dissolved in deuteriumn oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate(DSS) or sodium 3-(trimethylsilyl)propionate-d4(TSP) is used. For other nuclei, nitromnethane, trichlorofuoromethane and phosphoric acid are used as reference compounds for ^{15}N, ^{19}F and ^{31}P, respectively. Furthermore, chemical shifts of residual protons in deuterated solvents and ^{13}C in the solvent instead of a reference compound can be used for ^{1}H and ^{13}C NMR.

Record of apparatus and measurement conditions

Type of instrument, frequency, solvent, temperature, sample concentration, reference compound, experimental technique, etc. are recorded to allow appropriate comparison of spectra, because NMR spectra depend on the measurement conditions.

Identifications

The test solution is prepared and tested by the method directed in each monograph. Usually in the case of 1H NMR, the sample is identified by the following method.

(i) *Identification by the use of chemical shift, multiplicity and relative intensity*

When chemical shifts, multiplicities and relative intensities of signals are defined, the sample can be identified as being the same substance when all chemical shifts, multiplicities and relative intensities are the same as those prescribed.

(ii) *Identification by the use of a Reference Standard*

Measurement conditions is the same as those used in the case of the Reference Standard. When the spectra of a sample and the Reference Standard exhibit the same muliplicities and relative intensities of signal at the same chemical shifts, the sample can be identified as being the same substance as the Reference Standard.

Experimental techniques of ^{1}H and ^{13}C NMR spectroscopy

NMR spectroscopy includes one-, two- and multidimensional techniques, which are used for various purposes. Spin decoupling, and NOE are available in one-dimensional ^{1}H spectroscopy. Spin decoupling can assign coupling correlations. As NOE can observe correlations among spatially proximate protons, the configuration and the conformation can be analyzed.

Broadband decoupling, INEPT and DEPT are usually applied in one-dimensional ^{13}C spectroscopy. The broadband decoupling technique simplifies a spectrum and achieves enhancement of sensitivity. INEPT (insensitive nuclei enhanced by polarization transfer) and DEPT (distortionless enhancemment of polarization transfer) enhance the sensitivity of ^{13}C by means of polarization transfer from directly bonded 1H with a large magnetic moment. They can be applied to identify primary, secondary, tertiary or quarternary carbon.

Two-dimensional spectroscopy can observe all correlation peaks between nuclei through spin-spin coupling or NOE in a single experiment, and there are many techniques for homonuclear and heteronuclear measurements. Representative techniques are described below.

COSY (2D correlation spectroscopy), HOHAHA (homonuclear Hartmann-Hahn spectroscopy) or TOCSY (total correlation spectroscopy): Correlation between protons through scalar spin-spin coupling is obtained and intramolecular connectivities of hydrogen atoms are revealed.

NOESY (2D nuclear Overhauser enhancement and exchange spectroscopy): NOE is measured by a twodimensional technique. Approximate distances between spatially proximate hydrogen atoms are obtained to analyze the three-dimensional structure.

INADEQUATE (incredible natural abundance double quantum transfer experiment): Although this technique is insensitive because it involves double quantum transfer by ^{13}C-^{13}C scalar coupling in a sample with natural isotopic abundance, the connectivities of all neighboring ^{13}C nuclei can be obtained to analyze the carbon skeleton.

HMQC (heteronuclear multiple quantum coherence): This technique observes correlations between 1H and 13C with direct spin-spin coupling using 1H detection and reveals intramolecular chemical bonds between hydrogen and carbon atoms.

HMBC (heteronuclear multiple bond connectivity): This technique observes correlations betwcen 1H and 13C with long range spin-spin coupling using 1H detection and reveals intramoletiular connectivities of hydrogen and carbon atoms.

There are many other techniques such as DQFCOSY (double quantum filtered COSY) and HSQC (heteronuclear single quantum coherence). Furthermore, multidimensional NMR techniques are used to analyze macromolecules.

37. Optical Rotation Determination

The Optical Rotation Determination is a method for the measurement of the optical rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In the case of ordinary light, the directions of the planes are unrestricted. In the case of plane polarized light, commonly designated as polarized light, however, the vibrations take place on only one plane that includes the direction of the beam (plane of polarization). Some drugs in the solid state or in solution have the property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical structure of the substance.

The extent of the optical rotation, expressed in degrees of rotation of the angle of the plane of polarized light caused by the optically active substance or its solution, is measured with a polarimeter. This value is proportional to the length of the polarimeter tube, and is related to the concentration of the solution, the temperature and the wavelength. The character of the rotation is indicated by placing a plus sign (+) for that which rotates the plane of the polarized light to the right, when facing the direction of the beam, referred to as dextrorotatory, or a minus sign (-) for that which rotates the plane to the left, referred to as levorotatory, before the number indicating the degrees of rotation, as like as +20° meaning 20° to the right, or -20° meaning 20° to the left.

The optical rotation, α_x^t, is that which is measured with specific monochromatic light of x(described in terms of the wavelength or the name) at a temperature of t °C. Usually the measurement is performed at 20 °C or 25 °C, with a polarimeter tube of 100 mm in length, and with the D line of sodium as the light source.

The specific optical rotation, $[\alpha]_x^t$,is represented by the following equation:

$$[\alpha]_x^t = \frac{100 \times \alpha}{l \times c}$$

t: Temperature of measurement,

x: Wavelength or the name of the specific monochromatic light of the spectrum used (in the case of the D line, denoted as D),

a: Angle, in degrees, of rotation of the plane of the polarized light,

l: The thickness of the layer of test solution, i.e., the length of the polarimeter tube (mm),

c: For the Korean Pharmacopoeia, the number of grams of a drug present in 1mL of the solution. When an intact liquid drug is used for determination, not in solution, *c* represents the density. However, unless otherwise specified, the specific gravity is used instead of the density.

The description, for example, "$[\alpha]_D^{20}$:Between -33.0 and -36.0° (1 g, after drying, water, 20mL, 100 mm)," in a monograph, indicates that the $[\alpha]_D^{20}$ is between -33.0° and -36.0° in the determination in which the substance is dried under the conditions described in the test for Loss on Drying, and about 1 g of the substance is accurately weighed, and dissolved by adding water to make exactly 20mL, then the solution is measured with a polarimeter tube, 100 mm in length.

38. Osmolarity Determination

Osmolarity Determination is a method for the measurement of the osmotic concentration of the sample from the extent of the congealing-point depression.

When a solution and a pure solvent are separated by semipermeable membrane, which allows a solvent to pass freely and prevents the passage of a solute, a part of the solvent passes into the solution compartment through the membrane. The pressure difference produced between the two compartments with the solvent migration through the membrane is defined as the osmotic pressure P (Pa). The osmotic pressure is a physical quantity depending on the total of the molecular species present, including neutral molecules and ions, and does not depend on the kind of solute. A solution property, such as osmotic pressure, freezing-point depression, boiling-point elevation, etc., which depends not on the kind of solute, but on the total number of all molecular species, is called a colligative property of a solution.

The osmotic pressure of a polymer solution can be measured directly as the hydrostatic pressure difference between two compartments separated by a semipermeable membrane, such as a cellulose membrane. However, there is not any appropriate semipermeable membrane applicable to a solution containing low molecular species. Though the osmotic pressure of such a solution cannot be measured directly, the direction and extent of solvent migration through biological membranes can be predicted from the total number of all molecular species present when the solution is placed under physiological conditions. Other colligative properties of solution such as freezing-point depression, boiling-point elevation, vapor-pressure depression, etc., can be directly obtained by observing changes of temperature and/or pressure, etc. These solution properties depend on the total number of particles in the solution in the same way as the osmotic pressure, and the molecular particle concentration is defined as the osmotic concentration. The osmotic concentration can be defined as osmolality (mol/kg) based on the mass or osmolarity (mol/L) based on the volume. In practice, the latter is more convenient. Unless otherwise specified, the freezing-point depression method is used to measure osmotic concentration.

The freezing-point depression method determines the osmolality (*m*) by means of the freezing-point depression, ΔT(°C) in the following equation, based on the phenomenon that the congealing point of the solution depresses when a solute is dissolved in a solvent.

$$\Delta T = K \cdot m$$

where *K* (1.86 °C kg/mol, when the solvent is water) is the molal freezing-point depression constant.

Since the constant *K*is defined on the basis of molality, the above equation gives osmolality, but in the dilute concentration range, this can be assumed to be numerically equal to the osmolarity, *c* (mol/L). Thus, the conventional osmolarity and the unit of osmole(Osm, osmol/L) are adopted in this test method. One mOsm is $1/1000$ of 1 Osm. One Osm means that the Avogadro number (6.022×10^{23}/mol) of species is contained in 1000mL of solution. Usually, the osmotic concentration is expressed in the unit of mOsm.

Apparatus

Usually, osmotic concentration is obtained by measuring the extent of the freezing-point depression.

The osmometer consists of a test tube holding a fixed volume of the test solution, a cooling bath with a temperature regulator, and a unit (a thermistor thermometer) measuring temperature electrically.

Procedure

A fixed volume of the test solution is used for each determination as indicated for the individual apparatus. The apparatus for measuring osmotic pressure (osmolar concentration) is first calibrated by the two-point calibration method. For the calibration, use two standard solutions for osmometer calibration which span the expected osmotic pressure range of a test solution. Other than the standard solutions for osmometer calibration in the Table below, water can be also used as a standard solution (0 mOsm) in the place of one of two standard solutions for osmometer calibration for

measuring an osmolar concentration not higher than 100 mOsm. In the next, after washing the test sample cell and the thermistor as indicated for the individual apparatus, determine the freezing-point of the test solution and calculate the osmolality from the relation of osmolar concentration and the freezing-point depression and it is assumed to be numerically equal to the osmolarity.

In the cases of osmolar concentration higher than 1000 mOsm, dilute the sample by adding water to prepare *n* times diluted test solution (1 in *n*)and determine the osmolarity of the diluted solution. In this case, it is stated that the calculated osmolarity for the sample is an apparent osmolarity obtained from the measured value of the *n* times diluted test solution multiplied by the dilution number. When the dilution method is applied, the dilution number is selected so that the expected osmolarity is nearly equal to that of physiological saline solution. In the cases of solid samples, prepare a test solution by dissolving the solid in the indicated solvent.

Suitability of the apparatus

When the test is repeated 6 times with one of the standard solutions, of which concentration is near that of the test solution, the relative standard deviation of the test is not more than 2.0 % and the deviation of the average from the indicated osmolarity is not more than 3.0 %. When the requirements are not met, the suitability test is repeated after the apparatus is calibrated by the two-point calibration method.

Preparation of the standard solution for osmometer calibration

Weigh exactly an amount indicated in the following table of sodium chloride (standard reagent), previously dried between 500 °C and 650 °C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve the weighed sodium chloride in exactly 100 g of water to make the corresponding osmolar standard solution.

Standard solution for osmometer calibration (millosmoles)	Mass of sodium chloride (g)
100	0.309
200	0.626
300	0.946
400	1.270
500	1.593
700	2.238
1000	3.223

Osmotic pressure ratio

The ratio of the osmotic pressure of the sample to that of the Isotonic Sodium Chloride Solution is termed the osmotic pressure ratio in this test and is used as a measure of isotonicity of a test solution. Since the osmolarity of the Isotonic Sodium Chloride Solution is assumed to be constant (286 mOsm), the osmotic pressure ratio of a test solution, of which osmolarity is c_T(mOsm), can be calculated by the following equation.

$$\text{Osmotic pressure ratio} = \frac{c_T}{c_S}$$

c_S: 286 mOsm

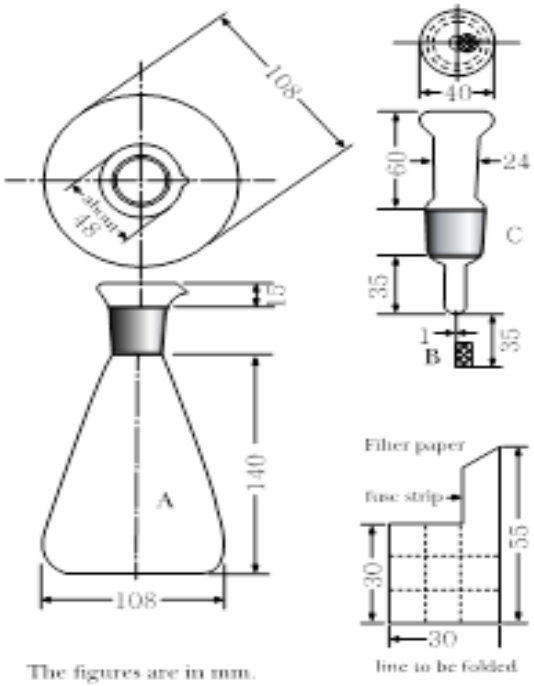
When the measurement is done by diluting the test solution, because the test solution has an osmolarity over 1000 mOsm, the apparent osmolarity of the test solution, c_T, can be calculated as $n \cdot c'_T = c_T$, in which *n* is the dilution factor and c'_T is the measured osmolarity for the diluted test solution. A linear relationship between osmolarity and solute concentration is assumed for this calculation. When the dilution is performed, the dilution factor is stated as (1 in *n*).

39. Oxygen Flask Combustion Method

The Oxygen Flask Combustion Method is a method for the identification or the determination of halogens or sulfur produced by combusting organic compounds, which contain chlorine, bromine, iodine, fluorine or sulfur, in a flask filled with oxygen.

Apparatus

Use the apparatus shown in the Figure.



A: Colorless, thick-walled (about 2 m), 500mL hard glass flask, the upper part of which is made like a saucer. A flask made of quartz should be used for the determination of fluorine

B: Platinum basket or cylinder made of platinum woven gauze. (It is hung at the end of the stopper C with platinum wire)

C: Ground stopper made of hard glass. A stopper made of quartz should be used for the determination of fluorine.

Preparation of test solution and blank solution
Unless otherwise specified, prepare them by the following method.

(1) ***Preparation of sample*** (i) *For solid samples* Place the quantity of the sample specified in the monograph on the center of the filter illustrated in the figure, weigh accurately, wrap the sample carefully along the dotted line without scattering, and place the parcel in a platinum basket or cylinder B, leaving its fuse-strip on the outside.

(ii) *For liquid samples* Roll a suitable amount of absorbent cotton with filter paper, 50 mm in length and 5 mm in width, so that the end part of the paper is left to a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder, B. Place the sample in a suitable glass tube, weigh accurately, and moisten the cotton with the quantity of the sample specified in the monograph, bringing the edge of the sample in contact with the cotton.

(2) ***Method of combustion*** Place the absorbing liquid specified in the monograph in flask A, fill it with oxygen, moisten the ground part of the stopper C with water, then ignite the fuse-strip, immediately transfer it to the flask, and keep the flask airtight until the combustion is completed. Shake the flask occasionally until the white smoke in A vanishes completely, allow to stand for 15 to 30 minutes, and designate the resulting solution as the test solution. Prepare the blank solution in the same manner, without sample.

Procedure of determination
Unless other-wise specified in the monograph, perform the test as follows.

(1) ***Chlorine and bromine*** Apply a small amount of water to the upper part of A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B, and the inner side of A with 15 mL of 2-propanol, and combine the washings with the test solution. To this solution, add 1 drop of bromophenol blue TS, add dilute nitric acid drop-wise until a yellow color develops, then add 25mL of 2-propanol, and titrate with 0.005 mol/L silver nitrate VS according to the potentiometric titration under the Endpoint Detection Methods in Titrimetry. Perform the test with the blank solution in the same manner, and make any necessary correction.

EachmL of 0.005 mol/L silver nitrate VS
= 0.17727 mg of Cl
EachmL of 0.005 mol/L silver nitrate VS
= 0.39952 mg of Br

(2) ***Iodine*** Apply a small amount of water to the upper part of A, pull out C carefully, add 2 drops of hydrazine hydrate to the test solution, put C on A, and decolorize the solution by vigorous shaking. Transfer the content of A to a beaker, wash C, B, and the inner side of A with 25mL of 2-propanol, and transfer the washings to the above beaker. To this solution, add 1 drop of bromophenol blue TS, then add dilute nitric acid drop–wise until a yellow color develops, and titrate with 0.005 mol/L silver nitrate VS according to the Potentiometric tiration under the Endpoint Detection Methods in Titrimetry. Perform the test with the blank solution in the same manner, and make any necessary correction.

EachmL of 0.005 mol/L silver nitrate VS
= 0.6345 mg of I

(3) ***Fluorine*** Apply a small amount of water to the upper part of A, pull out C carefully, transfer the test solution and the blank solution to 50 mL volumetric flasks separately, wash C, B, and the inner side of A with water, add the washings and water to make 50 mL, and use these solutions as the test solution and the correction solution. Pipet the test solution (V mL) equivalent to about 0.03 mg of fluorine, V mL of the correction solution and 5 mL of standard fluorine solution, transfer to 50 mL volumetric flasks separately, add 30 mL of a solution of pH 4.3 alizarin complexone TS in a mixture of acetic acid, potassium acetate buffer solution, and cerium(III)nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Prepare a blank with 5 mL of water in the same manner. Determine the absorbances. A_T , A_C and A_S , of the subsequent solutions of the test solution, the correction solution, and the standard solution at 600 nm, respectively in reference with the blank.

Amount (mg) of fluorine (F) in the test solution
= amount (mg) of fluorine in 5mL of
the standard solution $\times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$

Standard fluorine solution–Dry sodium fluoride (standard reagent) in a platinum crucible between 500 °C and 550 °C for 1 hour, cool it in a desiccator (silica gel), weigh accurately about 66.3 mg of it, and dissolve in water to make exactly 500 mL. Pipet 10 mL of this solution, and dilute with sufficient water to make exactly 100 mL.

4) ***Sulfur*** Apply a small amount of water to the upper part of A, pull out C carefully, and wash C, B, and the inner side of 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, allow to stand for 10 minutes, add 0.15 mL of arsenazo III TS with a measuring pipet, and titrate with 0.005 mol/L sulfuric acid VS. Perform the test with the blank solution in the same manner, and make any necessary correction.

EachmL of 0.005 mol/L barium perchlorate VS

= 0.16033 mg of S

40. Particle Size Distribution Test for Preparations

Particle Size Distribution Test is a method to determine the particle size distribution of the Granules and the Powders.

Procedure

Unless otherwise specified, the test is done by the following methods. If necessary, can be determined seperately.

(1) *Granules* The granules are tested using sieves of No. 10 (1700 μm), No. 12 (1400 μm), and No. 42 (355 μm). The inside diameter of the sieves used for the test is 75 mm. Weigh accurately about 20.0 g of granules, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount of granules remaining on each sieve and in the receiving pan. The requirement is met when all of the granules pass through the No. 10 (1700 μm) sieve, not more than 5 % of total granules remain on the No. 12 (1400 μm) sieve, and not more than 15 % of total granules pass through the No. 42 (355 μm) sieve.

(2) *Powders* The powders are tested using No. 18 (850 mm), No. 30 (500 mm), and No. 200 (75 mm) sieves. The inside diameter of the sieves used for the test is 75 mm. Place 10.0 g of powders, accurately weighed, on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap gently at intervals. Weigh the amount of powders remaining on each sieve and in the receiving pan. The requirement is met when all of the powders pass through the No. 18 (850 μm) sieve and not more than 5 % of total powders remain on the No. 30 (500 μm) sieve. Powders with not more than 10 % of passing through the No. 200 (75 μm) sieve meet the requirements for the Fine Granules.

41. pH Determination

pH is defined as the common logarithm of the reciprocal of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally, it is used as a scale of hydrogen ion concentration of a test solution.

pH of a test solution is expressed as the following equation in relation to the pH of a standard solution (pHs), and can be measured by a pH meter using a glass electrode.

$$pH = pH_S + \frac{E - E_S}{2.3026\ RT/F}$$

pH_S: pH value of a standard pH solution,

E: Electromotive force (volt) on the combination of glass and reference electrodes in a test solution; the constitution of the cell is expressed by the following:

Glass electrode | test solution | reference electrode

E_S: Electromotive force (volt) on the combination of glass and reference electrodes in a standard pH solution; the constitution of the cell is expressed by the following:

Glass electrode | standard pH solution | reference electrode

R: Gas constant,
T: Absolute temperature, and
F: Faraday constant.

The values of 2.3026 *RT/F* (volt) represent electromotive force (V) per pH unit and have temperature dependence as shown in the Table 1.

Table 1. Temperature dependence of electromotive force.

Temperature of solution (°C)	2.3026 RT/F	Temperature of solution (°C)	2.3026 RT/F
5	0.05519	35	0.06114
10	0.05618	40	0.06213
15	0.05717	45	0.06313
20	0.05817	50	0.06412
25	0.05916	55	0.06511
30	0.06015	60	0.06610

Standard pH solution

Standard pH solutions are used as standards of pH. To prepare the standard pH solutions, distill purified water or water with a conductivity not more than 2 μS·cm-1 (25 °C) and an organic carbon not more than 0.50 mg/L, boil the distillate for more than 15 minutes to expel carbon dioxide, and cool in a container fitted with a carbon dioxide-absorbing tube (soda lime). Prepare standard pH solutions of Table 2 as specified below. Store the standard pH solutions in hard glass or polyethylene bottles. Store basic standard solutions in containers fitted with a carbon dioxide-absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to acertain whether the expected pH is held or not by comparison with newly prepared standard, when the solution is used after a long period of storage.

(1) *Oxalate standard pH solution* Reduce potassium trihydrogen dioxalate dehydrate for pH determination to a fine powder, and dry in a desiccator (silica

gel). Weigh 12.71 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(2) *Phthalate standard pH solution* Reduce potassium hydrogen phthalate for pH determination to a fine powder, and dry at 110 °C to a constant mass. Weigh 10.21 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(3) *Phosphate standard pH solution* Reduce potassium dihydrogen phosphate for pH determination and anhydrous disodium hydrogen phosphate for pH determination to fine powders, and dry at 110 °C to a constant mass. Weigh 3.40 g (0.025 mole) of potassium dihydrogen phosphate and 3.55 g (0.025 mole) of disodium hydrogen phosphate accurately, and dissolve in water to make exactly 1000 mL.

(4) *Borate standard pH solution* Allow sodium borate for pH determination to stand in a desiccator (sodium bromide moistened with water) to a constant mass. Weigh 3.81 g (0.01 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(5) *Carbonate standard pH solution* Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to a constant mass, and weigh 2.10 g (0.025 mole) of it accurately. Dry sodium carbonate for pH determination between 300 °C and 500 °C to a constant mass, and weigh 2.65 g (0.025 mole) of it accurately. Dissolve both reagents in water to make exactly 1000 mL

(6) *Calcium hydroxide standard pH solution* Reduce calcium hydroxide for pH determination to a fine powder, transfer 5 g to a flask, add 1000mL of water, shake well, maintain the temperature between 23 °C and 27 °C, saturate thoroughly, and filter the supernatant liquid at the same temperature. Use the clear filtrate (about 0.02 mol/L).

The pH values of these pH solutions at various temperatures are shown in the Table 2. pH values at temperatures , which are listed in the table, are calculated by the interpolation.

Table 2. The temperature dependence of pH values of standard pH solutions.

Temperature(°C)	Oxalate pH standard solution	Phthalate pH standard solution	Phosphate pH standard solution
0	1.67	4.01	6.98
5	1.67	4.01	6.95
10	1.67	4.00	6.92
15	1.67	4.00	6.90
20	1.68	4.00	6.88
25	1.68	4.01	6.86
30	1.69	4.01	6.85
35	1.69	4.02	6.84
40	1.70	4.03	6.84
50	1.71	4.06	6.83
60	1.73	4.10	6.84
Temperature (°C)	Borate pH standard solution	Carbonate pH standard solution	Calcium hydroxide pH standard solution
0	9.46	10.32	13.43
5	9.39	10.25	13.21
10	9.33	10.18	13.00
15	9.27	10.12	12.81
20	9.22	10.07	12.63
25	9.18	10.02	12.45
30	9.14	9.97	12.30
35	9.10	9.93	12.14
40	9.07		11.99
50	9.01		11.70
60	8.96		11.45

Apparatus

A pH meter generally comprises a detection unit consisting of a glass electrode and a reference electrode, an amplifier amplifying the detected electromotive force and an display unit for indicating the measured results. For the display unit, dials for zero point adjustment and temperature compensation are fitted. Sometimes a dial for sensitivity adjustment are provided.

The reproducibility of a pH meter should be within 0.05 pH unit, when measurement of the pH value of one of the above standard solution is repeated five times by the following procedure, in which the detecting unit is washed well with water every time.

Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement more than 5 minutes after switching on. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

For the standardization of the pH meter, two pH standard solutions are usually used as follows. Immerse the detection unit in the phosphate pH standard solution and adjust the indicated pH to the pH value shown in the Table 2. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the test solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same conditions. Adjust the indicated pH to the defined pH value using the span adjustment dial, when the observed pH is not identical with that tabulated. Repeat the above standardization procedure until both pH standard solutions give observed pH values within 0.05 pH unit of the tabulated value without further adjustment. When a pH meter equipped with a temperature compensation dial, the standardization procedure is done after the setting of the temperature to that of the pH standard solution to be measured. In cases of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of the two pH standard solutions are identical with the tabulated values within 0.05 pH unit. After finishing the standardization procedure, rinse well the electrodes with water, and remove the attached water using a filter paper. Immerse the detection unit in the test solution and read the indicated pH value after confirming the value is stable. If necessary, a test solution can be agi-

tated gently. The temperature of test solution must be controlled to be the same (within 2 °C) as that of the pH standard solutions with which the pH meter was standardized. When a test solution is alkaline, the measurement should be done in a vessel with a cover and if necessary, in a stream of inert gas such as nitrogen. Furthermore, for a strongly alkaline solution above pH 11 containing alkaline metal ions, an alkali error may be induced. In such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

42. Pyrogen Test

The pyrogen test is a method to test the existence of pyrogens using rabbits.

Test animals

Use healthy mature rabbits, each weighing not less than 1.5 kg, which have not lost body mass when kept on a constant diet for not less than one week. House the rabbits individually in an area free from disturbances likely to excite them. Keep the temperature of the area constant between 20 °C and 27 °C for at least 48 hours before and throughout the test. Before using a rabbit that has not previously been used for a pyrogen test, condition it 1 to 3 days prior to the test by conducting a sham test omitting the injection. Do not use a rabbit for pyrogen tests more frequently than once every 48 hours, or after it has been given a test sample that was adjudged pyrogen-positive or that contained an antigen present commonly in the test sample to be examined .

Apparatus

(1) ***Thermometer*** Use a rectal thermometer or temperature-measuring apparatus with an accuracy of 0.1 °C or less.

(2) ***Syringes and injection needles*** Depyrogenate the syringes and needles in a hot-air oven using a validated process, usually by heating at 250 °C for not less than 30 minutes. Sterilized syringes with needles are also available provided that they have been validated to assure that they are free of detectable pyrogens and do not interfere with the test.

Procedures

(1) ***Volume of sample injection*** Unless otherwise specified, 10mL of the sample per kg of body weight of the animal is injected for the test.

(2) ***Procedure*** Perform the test in a separate area at an environmental temperature similar to that of the room wherein the animals were housed and free from disturbances likely to excite them. Withhold food from the rabbits for several hours before the first record of the temperature and throughout the testing period. The test animals are usually restrained with loosely fitting neck stocks that allow the rabbits to assume a natural resting posture. Determine the temperature of each rabbit by inserting the thermometer or temperature-measuring probe into the rectum of the test animal to a constant depth within the range of 60 mm to 90 mm. After the animal is stabilized, read the temperature and use it as a control temperature. Within 30 minutes of the reading, inject the test solution. During the injection, the difference between the temperature during the injection and that of the control should be 1 °C or less. Animals, which showed a control temperature of 39.8 °C or more, are excluded from the test. The test solution is warmed to 37 ± 2 °C and injected through the ear vein within 10 minutes per one animal. Hypotonic test sample may be made isotonic by the addition of pyrogen-free sodium chloride. Record the temperature of each rabbit during a period of 3 hours after the injection, taking the measurements at intervals of not more than 30 min. The difference between the control temperature and the highest temperature during the recording period is defined as the rise 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 zero. In case where no animal showed a temperature rise of 0.5 °C or more, the test result shall be considered negative for pyrogen and the sample considered acceptable. If one or more animal shows the temperature rise of 0.5 °C or more, the test is repeated. The second test is carried out in a group of 5 rabbits. If the number of the animals which showed the temperature rise of 0.5 °C or more is 3 or less, and if the sum of the temperature rise in the total 8 rabbits is 3.3 °C or less, the test result shall be considered negative for pyrogen.

43. Qualitative Tests

The Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5mL of the test solution.

Acetate

(1) When warmed with diluted sulfric acid (1 in 2), acetates evolve the odor of acetic acid.

(2) When an acetate is warmed with sulfuric acid and a small quantity of ethanol, the odor of ethyl acetate is evolved.

(3) Neutral solutions of acetates produce a red-brown color with iron (III) chloride TS, and a red-brown precipitate when boiled. The precipitate dissolves and the color of the solution changes to yellow upon addition of hydrochloric acid.

Aluminum salt

(1) Solutions of aluminum salts, when treated with ammonium chloride TS and ammonia TS, yield a gelatinous, white precipitate which does not dissolve in an excess of ammonia TS.

(2) Solutions of aluminum salts, when treated with sodium hydroxide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(3) Solutions of aluminum salts, when treated with sodium sulfide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(4) Add ammonia TS to solutions of aluminum salts until a gelatinous, white precipitate is produced. The color of the precipitate changes to red upon addition of 5 drops of alizarin S TS.

Ammonium salt

When heated with an excess of sodium hydroxide TS, ammonium salts evolve the odor of ammonia. This gas changes moistened red litmus paper to blue.

Antimony salt, Primary

(1) When primary antimony salts are dissolved in a slight excess of hydrochloric acid for the test and then diluted with water, a white turbidity is produced. The mixture produces an orange precipitate upon addition of 1 to 2 drops of sodium sulfide TS. When the precipitate is separated, and sodium sulfide TS is added to one portion of the precipitate and sodium hydroxide TS is added to another portion, it dissolves in either of these reagents.

(2) Add water to acidic solutions of primary antimony salts in hydrochloric acid until a small quantity of precipitate is produced, and then add sodium thiosulfate TS: the precipitate dissolves. A red precipitate is reproduced when the solution is heated.

Aromatic amines, Primary

Acidic solutions of primary aromatic amines, when cooled in ice, mixed with 3 drops of sodium nitrite TS under agitation, allowed to stand for 2 minutes, mixed well with 1mL of ammonium sulfamate TS, allowed to stand for 1 minute, and then mixed with ImL of N-(1-naphthyl)-N'-diethyl-ethylenediamine oxalate TS: the solution exhibits a red-purple color.

Arsenate

(1) Neutral solutions of arsenates produce no precipitate with 1 to 2 drops of sodium sulfide TS, but produce a yellow precipitate with hydrochloric acid subsequently added. The separated precipitate dissolves in ammonium carbonate TS.

(2) Neutral solutions of arsenates produce a dark red-brown precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia TS is add to another portion, the precipitate dissolves in either of these reagents.

(3) Neutral or ammonia alkaline solutions of arsenates produce with magnesia TS a white, crystalline precipitate, which dissolves in dilute hydrochloric acid subsequently added.

Arsenite

(1) Acidic solutions of arsenites in hydrochloric acid produce a yellow precipitate with 1 to 2 drops of sodium sulfide TS. When hydrochloric acid is added to one portion of the separated precipitate, it does not dissolve. When ammonium carbonate TS is added to another portion, the precipitate dissolves.

(2) Slightly alkaline solutions of arsenites produce a yellowish white precipitate with silver nitrate TS. When ammonia TS is added to one portion of the suspension, and dilute nitric acid is added to another portion, the precipitate dissolves in either of these reagents.

(3) Slightly alkaline solutions of arsenites produce a green precipitate with copper (II) sulfate TS. When the separated precipitate is boiled with sodium hydroxide TS, it changes to red-brown.

Barium salt

(1) When the Flame Coloration Test (1) is applied to barium salts, a persistent yellow-green color develops.

(2) Solutions of barium salts produce a white precipitate with dilute sulfuric acid. The precipitate does not dissolve in dilute nitric acid subsequently added.

(3) Acidic solutions of barium salts in acetic acid produce a yellow precipitate with potassium chromate TS. The precipitate dissolves in dilute nitric acid subsequently added.

Benzoate

(1) Concentrated solutions of benzoates produce a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed with cold water and dried, melts between 120 °C and 124 °C.

(2) Neutral solutions of benzoates produce a pale yellow-red precipitate upon drop-wise addition of iron (III) chloride TS. The precipitate changes to white on subsequent addition of dilute hydrochloric acid.

Bicarbonate

(1) Bicarbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with carbonates).

(2) Solutions of bicarbonates produce no precipitate with magnesium sulfate TS, but produce a white precipitate when boiled subsequently.

(3) A cold solution of bicarbonates remains unchanged or exhibits only a slightly red color upon addition of 1 drop of phenolphthalein TS (discrimination from carbonates).

Bismuth salt

(1) Bismuth salts, dissolved in a slight excess of hydrochloric acid, yield a white turbidity upon dilution with water. A dark brown precipitate is produced with 1 to 2 drops of sodium sulfide TS subsequently added.

(2) Acidic solutions of bismuth salts in hydrochloric acid exhibit a yellow color upon addition of thiourea TS.

(3) Solution of bismuth salts in dilute nitric acid or in dilute sulfuric acid yield a black precipitate with potassium iodide TS. The precipitate dissolves in an

excess of the reagent to give an orange-colored solution.

Borate

(1) A mixture prepared by addition of sulfuric acid and methanol to a borate burns, when ignited, with a green flame.

(2) Turmeric paper, when moistened with acidic solutions of borates in hydrochloric acid and dried by warming, exhibits a red color, which changes to blue with ammonia TS added drop-wise.

Bromate

(1) Acidic solutions of bromates in nitric acid yield a white, crystalline precipitate with 2 to 3 drops of silver nitrate TS. The precipitate dissolves upon heating. When 1 drop of sodium nitrite TS is added to this solution, a pale yellow precipitate is produced.

(2) Acidic solutions of bromates in nitric acid exhibit a yellow to red-brown color upon addition of 5 to 6 drops of sodium nitrite TS. When 1mL of chloroform is added to the mixture and shaken, the chloroform layer exhibits a yellow to red-brown color.

Bromide

(1) Solutions of bromides yield a pale yellow precipitate with silver nitrate TS. Upon addition of dilute nitric acid to a portion of the separated precipitate, it does not dissolve. When ammonia solution(28) is added to another portion and shaken, the separated solution yields a white turbidity upon acidifying with dilute nitric acid.

(2) Solutions of bromides exhibit a yellow-brown color with chlorine TS. The mixture is separated into 2 portions. When one portion is shaken with chloroform, the chloroform layer exhibits a yellow-brown to red-brown color. When phenol is added to the other portion, a white precipitate is produced.

Calcium salt

(1) When the Flame Coloration Test (1) is applied to calcium salts, a yellow-red color develops.

(2) Solutions of calcium salts yield a white precipitate with ammonium carbonate TS.

(3) Solutions of calcium salts yield a white precipitate with ammonium oxalate TS. The separated precipitate does not dissolve in dilute acetic acid, but dissolves in dilute hydrochloric acid subsequently added.

(4) Neutral solutions of calcium salts produce no precipitate, when mixed with 10 drops of potassium chromate TS and heated (discrimination from strontium salts).

Carbonate

(1) Carbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with bicarbonates).

(2) Solutions of carbonates yield a white precipitate with magnesium sulfate TS. The precipitate dissolves in dilute acetic acid subsequently added.

(3) Cold solutions of carbonates exhibit a red color with 1 drop of phenolphthalein TS (discrimination from bicarbonates).

Cerous salt

(1) When a cerous salt is mixed with 2.5 times its mass of lead (IV) oxide, nitric acid is added and the solution is boiled, it exhibits a yellow color.

(2) Solutions of cerous salts yield a yellow to red-brown precipitate upon addition of hydrogen peroxide TS and ammonia TS.

Chlorate

(1) Solutions of chlorates yield no precipitate with silver nitrate TS. When 2 to 3 drops of sodium nitrite TS and dilute nitric acid are added to the mixture, a white precipitate is produced gradually, which dissolves in ammonia TS subsequently added.

(2) When indigocarmine TS is added drop-wise to neutral solutions of chlorates until a pale blue color appears, and the mixture is acidified with dilute sulfuric acid, the blue color vanishes promptly upon subsequent drop-wise addition of sodium bisulfite TS.

Chloride

(1) Solution of chlorides evolve an odor of chlorine, when mixed with sulfuric acid and potassium permanganate, and heated. The gas evolved turns moistened potassium iodide starch paper blue.

(2) Solutions of chlorides yield a white precipitate with silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

Chromate

(1) Solutions of chromates exhibit a yellow color.

(2) Solutions of chromates produce a yellow precipitate with lead acetate TS. When acetic acid is added to a portion of the suspension, the precipitate does not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of chromates in sulfuric acid are mixed with an equal volume of ethyl acetate and 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed 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 or 2 drops of a solution of citrate, and the solution is allowed to stand for 2 to 3 minutes, a red-brown color develops.

(2) Neutral solutions of citrates, when mixed with an equal volume of dilute sulfuric acid and two-thirds volume of potassium permanganate TS, heated until the color of permanganate is discharged, and then

treated drop-wise with bromine TS to one-tenth of total volume, yield a white precipitate.

(3) Neutral solutions of citrates, when boiled with an excess of calcium chloride TS, yield a white, crystalline precipitate. When sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When dilute hydrochloric acid is added to another portion, the precipitate dissolves.

Cupric salt

(1) When a well polished iron plate is immersed in acidic solutions of cupric salts in hydrochloric acid, a red metallic film appears on its surface.

(2) Solutions of cupric salts produce a pale blue precipitate with a small quantity of ammonia TS. The precipitate dissolves in an excess of the reagent, yielding a deep-blue colored solution.

(3) Solutions of cupric salts yield a red-brown precipitate with potassium hexacyanoferrate (II) TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves, yielding a deep-blue colored solution.

(4) Solutions of cupric salts produce a black precipitate with sodium sulfide TS. When dilute hydrochloric acid, dilute sulfuric acid or sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When hot dilute nitric acid is added to another portion, the precipitate dissolves.

Cyanide

(1) Solutions of cyanides yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of cyanides yield a blue precipitate, when mixed by shaking with 2 to 3 drops of iron (II) sulfate TS, 2 to 3 drops of dilute iron (III) chloride TS and 1mL of sodium hydroxide TS, and then acidified with dilute sulfuric acid.

Dichromate

(1) Solutions of dichromates exhibit a yellow-red color.

(2) Solutions of dichromates produce a yellow precipitate with lead acetate TS. When acetic acid is added to one portion of the suspension, the precipitate dose not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of dichromates in sulfuric acid are mixed with an equal volume of ethyl acetate and with 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

Ferric salt

(1) Slightly acidic solutions of ferric salts yield a blue precipitate with potassium hexacyanoferrate (II) TS. The precipitate does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferric salts yield a gelatinous, red-brown precipitate with sodium hydroxide TS. The precipitate changes to black upon addition of sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid, yielding a white turbidity.

(3) Slightly acidic solutions of ferric salts exhibit a purple color with sulfosalicylic acid TS.

Ferricyanide

(1) Solutions of ferricyanides exhibit a yellow color.

(2) Solutions of ferricyanides yield a blue precipitate with iron (II) sulfate TS. The precipitate does not dissolve in dilute hydrochloric acid subsequently added.

Ferrocyanide

(1) Solutions of ferrocyanides yield a blue precipitate with iron (III) chloride TS. The precipitate does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrocyanides yield a red-brown precipitate with copper (II) sulfate TS. The precipitate does not dissolve in dilute hydrochloric acid subsequently added.

Ferrous salt

(1) Slightly acidic solutions of ferrous salts yield a blue precipitate with potassium hexacyanoferrate (III) TS. The precipitate does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrous salts yield a greenish gray, gelatinous precipitate with sodium hydroxide TS. The precipitate changes to black with sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid.

(3) Neutral or slightly acidic solutions of ferrous salts exhibit an intense red color upon drop-wise addition of a solution of o-phenanthroline in ethanol (1 in 50).

Fluoride

(1) When solutions of fluorides are heated with chromic acid-sulfuric acid TS, the inside of the test tube is not moistened uniformly.

(2) Neutral or slightly acidic solutions of fluorides exhibit a blue-purple color after standing with 1.5mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS(1:1:1).

Glycerophosphate

(1) Solutions of glycerophosphates remain unaffected by addition of calcium chloride TS, but yield a precipitate when boiled.

(2) Solutions of glycerophosphates yield no precipitate with ammonium molybdate TS when cold, but yield a yellow precipitate when boiled for a long time.

(3) When glycerophosphates are mixed with an equal mass of powdered potassium hydrogen sulfate and heated gently over a free flame, the pungent odor of acrolein is evolved.

Iodide

(1) Solutions of iodides yield a yellow precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia solution(28) to another portion, the precipitates do not dissolve in either of these reagents.

(2) Acidic solutions of iodides exhibit a yellow-brown color with 1 to 2 drops of sodium nitrite TS and then yield a black-purple precipitate. The solutions exhibit a deep-blue color with starch TS subsequently added.

Lactate

Acidic solutions of lactates in sulfuric acid, when heated with potassium permanganate TS, evolve the odor of acetaldehyde.

Lead salt

(1) Solutions of lead salts yield a white precipitate with dilute sulfuric acid. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When sodium hydroxide TS is added to another portion and warmed, or when ammonium acetate TS is added to another portion, the precipitate dissolves.

(2) Solutions of lead salts yield a white precipitate with sodium hydroxide TS. The precipitate dissolves in an excess of sodium hydroxide TS, and yields a black precipitate upon subsequent addition of sodium sulfide TS.

(3) Acidic solutions of lead salts in dilute acetic acid yield a yellow precipitate with potassium chromate TS. The precipitate does not dissolve in ammonia TS but dissolves in sodium hydroxide TS subsequently added.

Lithium salt

(1) When the Flame Coloration Test (1) is applied to lithium salts, a persistent red color develops.

(2) Solutions of lithium salts yield a white precipitate with disodium hydrogen phosphate TS. The precipitate dissolves upon subsequent addition of dilute hydrochloric acid.

(3) Solutions of lithium salts yield no precipitate with dilute sulfuric acid (discrimination from strontium salts).

Magnesium salt

(1) Solutions of magnesium salts yield upon warming with ammonium carbonate TS a white precipitate, which dissolves in ammonium chloride TS. A white, crystalline precipitate is reproduced by subsequent addition of disodium hydrogen phosphate TS.

(2) Solutions of magnesium salts yield a white, gelatinous precipitate with sodium hydroxide TS. When iodine TS is added to one portion of the suspension, the precipitate develops a dark-brown color. When excess sodium hydroxide TS is added to another portion, the precipitate does not dissolve.

Manganese salt

(1) Solutions of manganese salts yield a white precipitate with ammonia TS. When silver nitrate TS is added to a portion of the suspension, the precipitate changes to black. When another portion is allowed to stand, the upper part of the precipitate exhibits a brownish color.

(2) Acidic solutions of manganese salts in dilute nitric acid exhibit a purple-red color with a small quantity of powdered sodium bismuthate.

Mercuric salt

(1) A copper plate is immersed in solutions of mercuric salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercurous salts).

(2) Solutions of mercuric salts yield a black precipitate with a small quantity of sodium sulfide TS. The precipitate dissolves in an excess of the reagent. The black precipitate is reproduced by subsequent addition of ammonium chloride TS.

(3) When potassium iodide TS is added drop-wise to neutral solutions of mercuric salts, a red precipitate is produced. The precipitate dissolves in an excess of the reagent.

(4) Acidic solutions of mercric salts in hydrochloric acid yield a white precipitate with a small quantity of stannous chloride TS. The precipitate changes to grayish black upon addition of an excess of the reagent.

Mercurous salt

(1) A copper plate is immersed in solutions of mercurous salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercuric salts).

(2) Mercurous salts or their solutions exhibit a black color with sodium hydroxide TS.

(3) Solutions of mercurous salts yield a white precipitate with dilute hydrochloric acid. The separated precipitate changes to black upon addition of ammonia TS.

(4) Solutions of mercurous salts yield a yellow precipitate with potassium iodide TS. The precipitate changes to green, when allowed to stand, and changes again to black upon subsequent addition of an excess of the reagent.

Mesilate

(1) To mesilates add twice its mass of sodium hydroxide, heat gently to melt, and continue heating for 20 to 30 seconds. After cooling, add a little amount of water, then add dilute hyudrocholric acid, and warm: the gas evolved changes moistened potassium iodate-starch paper to blue.

(2) To mesilates add threefold its mass of sodium nitrate and anhydrous sodium carbonate, mix, and heat gradually. After cooling, dissolve the residue in diluted hydrochloric acid (1 in 5), and filter if necessary. The filtrate yields a white precipitate upon addition of barium chloride TS.

Nitrate

(1) When a solution of nitrates is mixed with an equal volume of sulfuric acid, the mixture is cooled, and ferrous sulfate TS is superimposed, a dark-brown ring is produced at the junction of the two liquids.

(2) Solutions of nitrates exhibit a blue color with diphenylamine TS.

(3) When potassium permanganate TS is added to acidic solutions of nitrates in sulfuric acid, the red-purple color of the reagent does not fade (discrimination from nitrites).

Nitrite

(1) Solutions of nitrites, when acidified with dilute sulfuric acid, evolve a yellow-brown gas with a characteristic odor. The solutions exhibit a dark-brown color upon addition of a small quantity of iron (II) sulfate heptahydrate crystals.

(2) Solutions of nitrites, when 2 to 3 drops of potassium iodide TS and dilute sulfuric acid are added drop-wise, exhibit a yellow-brown color, and then yield a black-purple precipitate. When the mixture is shaken with 2mL of chloroform, the chloroform layer exhibits a purple color.

(3) Solutions of nitrites, when mixed with thiourea TS and acidified with dilute sulfuric acid, and iron(III)chloride TS is added drop-wise, exhibit a dark red color. When the mixture is shaken with 2mL of ether, the ether layer exhibits a red color.

Oxalate

(1) When potassium permanganate TS is added drop-wise to warm acidic solutions of oxalates in sulfuric acid, the reagent is decolorized.

(2) Solutions of oxalates yield a white precipitate with calcium chloride TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

Permanganate

(1) Solutions of permanganates exhibit a red-purple color.

(2) When an excess of hydrogen peroxide TS is added to acidic solutions of permanganates in sulfuric acid, the solutions effervesce and decolorize permanganates.

(3) Acidic solutions of permanganates in sulfuric acid are decolorized, when an excess of oxalic acid TS is added and heated.

Peroxide

(1) Solutions of peroxides are mixed with an equal volume of ethyl acetate and 1 to 2 drops of potassium dichromate TS, and then acidified with dilute sulfuric acid. When the mixture is shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

(2) Acidic solutions of peroxides in sulfuric acid decolorize potassium permanganate TS, added drop-wise, and effervesce to evolve a gas.

Phosphate (Orthophospbate)

(1) Neutral solutions of phosphates yield a yellow precipitate with silver nitrate TS. The precipitate dissolves upon addition of dilute nitric acid or ammonia TS.

(2) Neutral solutions or acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with ammonium molybdate TS on warming. The precipitate dissolves upon subsequent addition of sodium hydroxide TS or ammonia TS.

(3) Neutral or ammonia-alkaline solutions of phosphates yield a white, crystalline precipitate with magnesia TS. The precipitate dissolves upon subsequent addition of dilute hydrochloric acid.

Potassium salt

(1) When the Flame Coloration Test (1) is applied to potassium salts, a pale purple color develops. When it gives a yellow color, a red-purple color can be seen through cobalt glass.

(2) Neutral solutions of potassium salts yield a white, crystalline precipitate with sodium bitartrate TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod. The separated precipitate dissolves upon addition of any of ammonia TS, sodium hydroxide TS or sodium carbonate TS.

(3) Acidic solutions of potassium salts in acetic acid yield a yellow precipitate with sodium cobalt nitrite TS.

(4) Potassium salts do not evolve the odor of ammonia, when an excess of sodium hydroxide TS is added and warmed (discrimination from ammonium salts).

Salicylate

(1) Salicylates evolve the odor of phenol, when an excess of soda-lime is added and heated.

(2) Concentrated solutions of salicylates yield a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed well with cold water and dried, melts at about 159 °C.

(3) Neutral solutions of salicylates exhibit with 5 to 6 drops of dilute iron (III) chloride TS a red color, which changes to purple and then fades when dilute hydrochloric acid is added drop-wise.

Silver salt

(1) Solutions of silver salts yield a white precipitate with dilute hydrochloric acid. When dilute nitric acid is added subsequently to a portion of the suspension, the precipitate does not dissolve. When an excess

of ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of silver salts yield a red precipitate with potassium chromate TS. The precipitate dissolves upon addition of dilute nitric acid.

(3) Solutions of silver salts yield a brownish gray precipitate with ammonia TS added drop-wise. When ammonia TS is added drop-wise until the precipitate dissolves, then 1 to 2 drops of formaldehyde solution are added and warmed, a mirror of metallic silver is deposited on the inside wall of the container.

Sodium salt

(1) When the Flame Coloration Test (1) is applied to sodium salts, a yellow color develops.

(2) Concentrated, neutral or slightly alkaline solutions of sodium salts yield a white, crystalline precipitate with potassium hexahydroxoantimonate(V) TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

Stannic salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannic salts in hydrochloric acid and is placed in a non-luminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannous salts).

(2) When granular zinc is immersed in acidic solutions of stannic salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannous salts).

(3) Add iron powder to acidic solutions of stannic salts in hydrochloric acid, allow to stand, and then filter. When iodine-starch TS is added drop-wise to the filtrate, the color of the test solution disappears.

(4) Acidic solutions of stannic salts in hydrochloric acid, to which ammonia TS is added drop-wise until a small quantity of precipitate is produced, yield a pale yellow precipitate with 2 to 3 drops of sodium sulfide TS. The separated precipitate dissolves upon addition of sodium sulfide TS and pale yellow precipitate is reproduced by subsequent addition of hydrochloric acid.

Stannous salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannous salts in hydrochloric acid and is placed in a non-luminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannic salts).

(2) When granular zinc is immersed in acidic solutions of stannous salts 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 drop-wise to solutions of stannous salts, the color of the test solution disappears.

(4) Acidic solutions of stannous salts in hydrochloric acid, to which ammonia TS is added drop-wise until a small quantity of precipitate is produced, yield a dark brown precipitate with 2 to 3 drops of sodium sulfide TS. When sodium sulfide TS is added to a portion of the separated precipitate, it does not dissolve. When ammonium polysulfide TS is added to another portion, the precipitate dissolves.

Sulfate

(1) Solutions of sulfates yield a white precipitate with barium chloride TS. The precipitate does not dissolve upon addition of dilute nitric acid.

(2) Neutral solutions of sulfates yield a white precipitate with lead acetate TS. The precipitate dissolves upon subsequent addition of ammonium acetate TS.

(3) When an equal volume of dilute hydrochloric acid is added, solutions of sulfates yield no white turbidity (discrimination from thiosulfates), and do not evolve the odor of sulfur dioxide (discrimination from sulfites).

Sulfide

Most kinds of sulfides evolve the odor of hydrogen sulfide with dilute hydrochloric acid. This gas blackens lead acetate paper moistened with water.

Sulfite and Bisulfite

(1) When iodine TS is added drop-wise to acidic solutions of sulfites or bisulfites in acetic acid, the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of sulfites or bisulfites evolve the odor of sulfur dioxide but yield no turbidity (discrimination from thiosulfates). The solutions yield immediately with 1 drop of sodium sulfide TS a white turbidity, which changes gradually to a pale yellow precipitate.

Tartrate

(1) Neutral tartrate solutions yield a white precipitate with silver nitrate TS. When nitric acid is added to a portion of the separated precipitate, it dissolves. When ammonia TS is added to another portion and warmed, the precipitate dissolves and metallic silver is deposited gradually on the inside wall of the test tube, forming a mirror.

(2) Solutions of tartrates exhibit a red-purple to purple color, when 2 drops of acetic acid(31), 1 drop of iron (II) sulfate TS, 2 to 3 drops of hydrogen peroxide TS and an excess of sodium hydroxide TS are added.

(3) When 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 5mL of sulfuric acid, is added to 2 to 3 drops of solutions of tartrates, and then heated for 5 to 10 minutes on a water-bath, a deep blue color is produced. The solution exhibits a red to red-orange color when poured to 3mL of water after cooling.

Thiocyanate

(1) Solutions of thiocyanates yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When strong ammonia water is added to another portion, the precipitate dissolves.

(2) Solutions of thiocyanates produce a red color with iron(III)chloride TS. The color does not disappear by addition of hydrochloric acid.

Thiosulfate

(1) When iodine TS is added drop-wise to acidic solutions of thiosulfates in acetic acid, the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of thiosulfates evolve the odor of sulfur dioxide, and yield gradually a white turbidity, which changes to yellow on standing.

(3) Solutions of thiosulfates yield a white precipitate with an excess of silver nitrate TS. The precipitate changes to black on standing.

Zinc salt

(1) Neutral to alkaline solutions of zinc salts yield a whitish precipitate with ammonium sulfide TS or sodium sulfide TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

(2) Solutions of zinc salts yield a white precipitate with potassium hexacyano ferrate (II) TS.. When dilute hydrochloric acid is added to a portion of the suspension, the precipitate does not dissolve. When sodium hydroxide TS is added to another portion, the precipitate dissolves.

(3) Neutral to weakly acidic solutions of zinc salts yield a white precipitate, when 1 or 2 drops of pyridine and 1mL of potassium thiocyanate TS are added.

44. Readily Carbonizable Substances Test

The Readily Carbonizable Substances Test is a method to examine the minute impurities contained in drugs, which are readily colored by addition of 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, place 5 mL of sulfuric acid for readily carbonizable substances in a Nessler tube, to which add a 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, transfer a volume of the sample, as directed 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 of the tube rises, cool the content; maintain it at the standard temperature, if the reaction may be affected by the temperature. Allow to stand for 15 minutes, and compare the color of the liquid with that of the matching fluid in the Nessler tube specified in the monograph, by viewing transversely against a white background.

45. Refractive Index Determination

The Refractive Index Determination is a method to measure the ratio of the velocity of light in air to that in the sample. Generally, when light proceeds from one medium into another, the direction is changed at the boundary surface. This phenomenon is called refraction.

When light passes from the first isotropic medium into the second, the ratio of the sine of the angle of incidence, i, to that of the angle of refraction, r, is constant with regard to these two media and has no relation to the angle of incidence. This ratio is called the refractive index of the second medium with respect to the first, or the relative refractive index, n.

$$n = \frac{\sin i}{\sin r}$$

The refractive index obtained with a vacuum as the first medium is called the absolute refractive index, N, of the second medium.

In isotropic substances, the refractive index is a characteristic constant at a definite wavelength, temperature, and pressure. Therefore, this measurement is applied to purity test of substances, or to determination of the composition of homogeneous mixtures of two substances.

The measurement is usually carried out at 20 °C and the D line of the sodium spectrum is used for irradiation. This value is expressed as n_D^{20}

Procedure

For the measurement of refractive index, usually the Abbe refractometer is used at a temperature in the range of ± 0.2 °C of that directed in the monograph. Use of the Abbe refractometer permits direct reading of $n_D$under incandescent light, with a measurable range from 1.3 to 1.7, and an attainable precision of 0.0002.

46. Residue on Ignition Test

The Residue on Ignition Test is a method to measure the mass of the residual substance not volati-

lized when the sample is ignited by the method described below.

Generally, this test is intended for determining the content of inorganic substances contained as impurities in an organic substance, and, occasionally, for determining the amount of inorganic substances contained as components in an organic substance, or the amount of impurities contained in a volatile inorganic substance.

The description, for example, "not more than 0.10 percent (1 g)," in a monograph, indicates that the mass of the residue is not more than 1.0 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and 『after drying』 indicates that the sample is subjected to the procedure after being dried under the conditions specified in the test for Loss on Drying.

Procedure

Previously ignite a crucible of platinum, quartz or porcelain to a constant mass at 600 ± 50 °C for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately after cooling.

Take the amount of the sample specified in the individual monograph in the crucible and weigh the crucible accurately .

Moisten the specimen with a small amount (usually 1mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount (usually 1mL) of sulfuric acid, heat gently until white fumes are no longer evolved, and ignite at 600 ± 50 °C until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of the residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening procedure with sulfuric acid, heating and ignition as before, using a 30 minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph .

47. Sterility Test

The test is applied to active pharmaceutical ingredients, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined in the condition of the test.

Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any microorganisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

Culture media and incubation temperatures

(1) *General requirement* Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test. The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soybean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

(2) *Fluid thioglycolate medium*

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate or glucose anhydrate	5.5 or 5.0 g
Yeast extract	5.0 g
Casein peptone	15.0 g
Sodium thioglycollate or thioglycolic acid	0.5 g or 0.3 mL
Resazurin solution (1 in 1000)	1.0 mL
Water	1000 mL
(pH after sterilization 7.1±0.2)	

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with water, and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution (1 in 1000), mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2 °C and 25 °C in a sterile, tight container. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30 – 35 °C. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20 – 25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.
Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30 – 35 °C under anaerobic conditions.

(3) *Soybean-casein digest medium*

Casein peptone	17.0 g
Soybean pepton	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphaye	2.5 g
Glucose monohydrate or glucose anhydrate	2.5 or 2.3 g
Water	1000 mL
(pH after sterilization 7.3 ± 0.2)	

Dissolve the solids in water, warming slightly to effect solution. Cool the solution to room temperature. Add sodium hydroxide TS, if necessary, so that after sterilization the solution will have a pH of 7.3 ± 0.2. Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2 °C and 25 °C in a sterile tight container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.
Soybean casein digest medium is to be incubated at 20 – 25 °C.

Suitability of media

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.
(1) *Sterility of media* Incubate portions of the media for 14 days. No growth of micro-organisms occurs.
(2) *Growth promotion test of aerobes, anaerobes and fungi* Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of micro organisms are indicated in Table 1.
Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of microorganism:
Clostridium sporogenes, Pseudomonas aeruginosa, Staphylococcus aureus.
Inoculate portion of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of microorganism:
Aspergillus brasiliensis, Bacillus subtilis, Candida albicans.
Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.
Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.
The media are suitable if a clearly visible growth of the microorganisms occurs.

Table 1. Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Method suitability Test

Aerobic bacteria	
Staplrylococcus 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	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455, KCTC 6317 and 6196

Method suitability test

Carry out a test as described below under 「Test for sterility of the product」 to be examined using exactly the same methods except for the following modifications.
(1) Membrane filtration: After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the final portion of sterile diluents used to rinse the filter.
(2) Direct inoculation: After transferring the contents of the container or containers to be tested to the culture medium add and inoculums of a small number of viable microorganisms (not more than 100 CFU) to the medium.
In both cases use the same microorganisms as those described above under 3.2. Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 3 days.
If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the condi-

tions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.
If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.
This method suitability is performed:
a) when the test for sterility has to be carried out on a new product;
b) whenever there is a change in the experimental conditions of the test.
The method suitability 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 requirement*
The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.
(2) *Membrane filtration*
Use membrane filters having a nominal pore size not greater than 0.45µm whose effectiveness to retain micro-organisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g., for antibiotics.
The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane 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 permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

(i) *Aqueous solutions*: If appropriate, transfer a small quantity of a suitable, sterile diluents such as a 1 g/L neutral solution of meat or casein peptone pH 7.1 ± 0.2 onto the membrane in the apparatus and filter. The diluents may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluents but in any case using not less than the quantities of the product to be examined prescribed in Table 2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluents used in the method suitability test. Do not exceed a washing cycle of 5 times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

Table 2. Minimum quantity to be used for each medium

Quantity per comtainer	Minimum quantity to be used for each medium unless otherwise justified and authorized
Liquids	
Less than 1 mL: 1-40 mL: Greater than 40 mL and not greater than 100 mL Antibiotic liquids	The whole contents of each container Half the contents of each contener but not less than 1 mL 20 mL 10 % of the contents of the container but nor less than 20 mL 1 mL
Insoluble preparations, creams and ointment to be suspended or emulsified	Use the contents of each container to provide not less than 0.2 g
Solide	
Less than 50 mg 50 mg or more but less than 300 mg 300 mg-5 g greater than 5 g	The whole contents of each container Half the contents of each container but not less than 50 mg 150 mg 150 mg

(ii) *Soluble solids*: Use for each medium not less than the quantity prescribed in Table 2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injection, saline or a

1 g/L neutral solution of meat or casein peptone and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

(iii) *Oils and oily solutions*: Use for each medium not less than the quantity of the product prescribed in Table 2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluents such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as 1 g/L neutral meat or casein peptone containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.

(iv) *Ointments and creams*: Use for each medium not less than the quantities of the product prescribed in Table 2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 % in isopropyl myristate as described above, by heating, if necessary, to not more than 40 °C. In exceptional cases it may be necessary to heat to not more than 44 °C. Filter as rapidly as possible and proceed as described above for oils 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 not more than 10 % of the volume of the medium, unless otherwise prescribed. If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

(i) *Oily liquids*: Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/L.

(ii) *Ointments and creams*: Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluents such as a 1 g/L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic microorganisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

Observation and interpretation of results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1mL) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 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 may be considered invalid only if one or more of the following conditions are fulfilled:

(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 test in question reveals a fault;

(c) microbial growth is found in the negative controls;

(d) after determination of the identity of the microorganisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

If the test is declared to be invalid it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with 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 technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 2, diluting where necessary to about 100 mL with a suitable sterile solution, such as 1 g/L neutral meat or casein peptone.

When using the technique of direct inoculation of media, use the quantities shown in Table 2, unless otherwise justified and authorized. The test for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the test, the contents of two or more containers are used to inoculate the different media.

Minimum number of items to be tested

The minimum number of items to be tested in relation to the size of the batch is given in Table 3.

Table 3. Minimum number of items to be tested

Numver of items in the batch*	Minimum number of items to be tested for each medium, unless otherwise justified and authorized**
Parenteral preparations	
Not more than 100 containers More than 100 but not more than 500 containers More than 500 comtainers	10 % or 4 containers whicheveris the greater 10 containers 2 % or 20 containers (10 containers for parenterals with a nominal volume of 100mL or more) whicheveris the less
Ophthalmic and other non-injectable preparations	
Not more than 200 containers More than 200 containers If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use	5 % or 2 containers whicheveris the greater 10 containers
Bulk solid products	
Up to 4 containers More than 4 containers but not more than 50 containers More than 50 containers	Each container 20 % or 4 containers whicheveris the greater 2 % or 10 containers whicheveris the greater

* If the batch size is not known, use the maximum numver of items prescribed

** If the contents of one containers are enough to inoculate the two media, this column gives the number of containers needed for voth media together

48. Sulfate Limit Test

The Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO4) is described in terms of percentage in parentheses.

Procedure

Unless otherwise specified, transfer a quantity of the sample as directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add 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. Transfer a volume of 0.005 mol/L sulfuric acid VS, as directed in the monograph, to another Nessler tube, add 1mL of dilute hydrochloric acid and water to make 50mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions under the same conditions.

Add 2 mL each of barium chloride TS to the test solution and the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

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

49. Test for Acid-neutralizing Capacity

The Test for Acid-neutralizing Capacity is a test to determine the acid-neutralizing capacity of a medicine, as a raw material or a preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a herbal material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

Preparation of sample

A raw material and a solid preparation which conforms to powders in the General Rules for Preparations: may be used, without any treatment, as a sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as a sample. Granules in unit-dose packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as a sample. Granules not in unit-dose packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately and powder not less than 20 doses, and use as a sample. Capsules and tablets: take not less than 20 doses, weigh accurately, calculate the average mass for a daily dose, powder it, and use as a sample. Liquid preparations: shake well, and use as a sample.

Procedure

Take an amount of the sample so that 'a' in the following equation falls between 20 mL and 30 mL, and perform the test. Accurately weigh the sample of the raw material or preparation, and place it in a glass-stoppered, 200 mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at

37 ± 2 °C for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination, end point: pH 3.5). Perform a blank determination, and make any necessary correction.

For liquid preparations, pipet the sample in a 100 mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.2 mol/L hydrochloric acid VS while shaking. Add water again to make the solution exactly 100 mL. Transfer the solution to a glass-stoppered, 200 mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at 37 ± 2 °C for 1 hour, and filter. Pipet 60mL of the filtrate, and titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination, end point: pH 3.5). Perform a blank determination, and make any necessary correction.

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

$$(\text{mL}) = \frac{(b-a)\times f\times 2\times \frac{t}{s}}{}$$

a: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed,

b: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination,

f: The molarity coeffcient of 0.1 mol/L sodium hydroxide VS,

t: 1000 mg of raw material or daily dose of preparation (in mg of solid preparation,mL of liquid preparation), and

s: Amount of the sample (in mg of raw material and solid preparation,mL of liquid preparation)

50. Test for Glass Containers for Injections

The glass containers for injections do not interact physically or chemically with the contained medicament to alter any property or quality, can protect the contained medicament from the invasion of microbes by means of perfect sealing or other suitable process, and meet the following requirements. The surface-treated container for aqueous infusion is made from glass which meets the requirements for the soluble alkali test for a container not to be fused under Method 1.

The containers are colorless or light brown and transparent, and have no bubbles which interfere with the test for foreign material specified in General Rules for Preparations, Injections (12).

Multiple-dose containers are closed by rubber stoppers or any other suitable stoppers. The stoppers permit penetration of an injection needle without detachment of fragments, and upon withdrawal of the needle, they reclose the containers immediately to prevent external contamination, and also do not interact physically or chemically with the contained medicaments.

Containers intended for aqueous infusions are closed by rubber stoppers meeting the requirements for Rubber Closure for Aqueous Infusions.

Soluble alkali test

The testing methods may be divided into the following two methods according to the type of container or the dosage form of the medicament.

(1) ***Method 1*** This method is applied to containers to be fused, or containers not to be fused except containers for aqueous infusions with a capacity exceeding 100mL. Rinse thoroughly the inside and outside of the containers to be tested with water, dry, and roughly crush, if necessary.

Transfer 30 to 40 g of the glass to a steel mortar, and crush. Sieve the crushed glass through a No. 12 (1400 μm) sieve. Transfer the portion retained on the sieve again to the steel mortar, and repeat this crushing procedure until 2/3 of the amount of powdered glass has passed through a No. 12 (1400 um) sieve. Combine all portions of the glass powder passed through a No. 12 (1400 um) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850 um) and No. 50 (300 um) sieves. Take 7 g of the powder, which has passed through a No. 18 (850 um) sieve but not through a No. 50 (300 um) sieve, immerse it in a suitable container filled with water, and wash the contents with gentle shaking for 1 minute. Rinse again with ethanol (95) for 1 minute, dry the washed glass powder at 100 °C for 30 minutes, and allow it to be cooled in a desiccator (silica gel). Transfer exactly 5.0 g of the resulting powder to a 250 mL Erlenmeyer flask of hard glass, add 50 mL of water, and gently shake the flask so that the powder disperses on the bottom of the flask evenly. Cover the flask with a small beaker of hard glass or a watch glass of hard glass, then heat it in boiling water for 2 hours, and immediately cool to room temperature. Decant the water from the flask into a 250 mL Erlenmeyer flask of hard glass, wash well the residual powdered glass with three 20mL volumes of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red purple. Perform a blank determination in the same manner, and make any necessary correction.

The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed the following quantity, according to the type of containers.

Containers to be fused	0.30 mL
Containers not to be fused (including injection used as containers)	2.00 mL

(2) ***Method 2*** This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, and dry. Add a volume of water equivalent to 90 % of the overflow capacity of the container, cover it with a small beaker of hard glass or close tightly with a suitable stopper, heat in an autoclave at 121 °C for 1 hour, and allow to stand until the temperature reaches to room temperature, measure exactly 100 mL of this solution, and transfer to a 250 mL Erlenmeyer flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Measure accurately 100 mL of water, transfer to a 250 mL Erlenmeyer flask of hard glass, perform a blank determination in the same manner, and make any necessary correction. The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed 0.10 mL.

Soluble iron test for light-resistant containers

Rinse thoroughly five or more light-resistant containers to be tested with water, and dry at 105 °C for 30 minutes. Pour a volume of 0.01 mol/L hydrochloric acid VS corresponding to the labeled volume of the container into individual containers, and fuse them. In the case of containers not to be fused, cover them with small beakers of hard glass or watch glasses of hard glass. Heat them at 105 °C for 1 hour. After cooling, prepare the test solution with 40mL of this solution according to Method 1 of the Iron Limit Test, and perform the test according to Method B. Prepare the control solution with 2.0 mL of the standard iron solution.

Light transmission test for light-resistant containers

Cut five light-resistant containers to be tested, prepare test pieces with surfaces as flat as possible, and clean the surfaces. Fix a test piece in a cell-holder of a spectrophotometer to allow the light pass through the center of the test piece perpendicularly to its surface. Measure the light transmittance of the test piece with reference to air between 290 nm and 450 nm and also between 590 nm and 610 nm at intervals of 20 nm each. The percent transmissions obtained between 290 nm and 450 nm are not more than 50 % and that between 590 nm and 610 nm are not less than 60 %. In the case of containers not to be fused having a wall thickness over 1.0 mm, the percent transmissions between 590 nm and 610 nm are not less than 45 %.

51. Test for Herbal Drugs

This method is applicable for testing all medicines containing herbal ingredients.

Sampling Unless otherwise specified, employ the following sampling method; and if necessary, store sample in a tight container.

A. Small amount: mix grounded or powdered herbal medicines and collect 50 ~ 250 g.

B. Large amount: mix herbal medicines and collect 250 ~ 500 g.

C. If each herbal medicine ingredient weighs 100g or more, mix 5 pieces of specimen or smaller and collect 500 g.

Preparation of sample for analysis Mix powdered or ground ssample, unless specified otherwise. For sampling that cannot be ground, cut it into smallest possible pieces and spread it thinly for collection.

Microscopic examination

Equipment Use an optical microscope: 10x, 40x object lenses and a 10x eyepiece.

Preparation of sample

Section Place a section on a glass slide and add 1 ~ 2 drops of mounting agent. Gently place a cover slip on top whilst avoiding trapping any air bubbles. Thickness of the sample section should be 10 ~ 20 μm.

Powder Place 1mg of sampling powder on a watch glass and add 1 ~ 2 drops of swelling agent. Mix using a glass rod, whilst avoiding trapping any air bubbles and then let it stand for 10 minutes to swell. Smear a small amount of the swollen sample onto a glass slide, using the tip of a glass rod, and add a drop of mounting agent. Spread it thinly to avoid particles of tissue from overlapping, and then place a cover slip gently on top, whilst 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.

Component observation In accordance with appropriate clauses for each relevant regulation for each drug and medication, the exterior, interior and tissue content section should be observed. Powdered ingredient should be observed in the order of special characteristics, commonly, rarely occurring characteristics and tissue content.

Purity

Foreign matter Unless otherwise specified, spread 25 ~ 500 g of herbal medicine thinly and eliminate any foreign matter identified by the naked eye or through a magnifying glass. Weigh the foreign matter and calculate percentage.

Heavy metals

Lead, arsenic and cadmium

Preparation of test solution

(1) Microwave method: Place 0.1 ~ 0.5 g of sampling powder into a microwave sample pre-treatment container and add 12mL of nitric acid. If the specimen does not dissolve, add 1 ~ 2 mL of hydrochloric acid or hydrogen peroxide (30). Remove the gas gener-

ated from this process by placing the container in the hood and break it down further using the microwave sample pre-treatment container.
When it is completely dissolved, filter the liquid into a volume flask using a filter paper and dilute it with water to the concentration similar to that of standard solution. Use this as test solution.
Place 12 mL of nitric acid into the microwave sample pre-treatment container and repeat the same process to prepare standard test solution.

(2) Wet combustion method: Place 2 ~ 5 g of the sampling powder into a beaker and add 10 ~ 30 mL of nitric acid. Cover it with watch glass and let it stand overnight. Place it on a heat plate and break it down gradually by increasing the heat until it stops generating brown smoke. If it does not break down, add 10mL of nitric acid. Add 5 ~ 10 mL of hydrogen peroxide (30) to dissolve it completely until it turns light yellow or yellow.
If it does not dissolve completely, use the microwave sample pre-treatment container to further breakdown. Concentrate this liquid on a heat plate to 1 ~ 2 mL and cool it. Filter the liquid into a volume flask using filter paper and dilute it with water to the concentration similar to that of standard solution. Use this as test solution.
To prepare test solution for measuring arsenic, add 1mL of potassium iodide test solution to 10mL of the test solution and then add 50 mL of 0.5 mol/L hydrochloric acid (skip this process if ICP-MS is used for the measurement).
Repeat this process with 10 ~ 30 mL of nitric acid to prepare standard test solution.

Measurement
Measurement can be made using test solution, standard solution and standard test solution by following the Atomic Absorption Spectrometric method. Using the Atomic Absorption Spectrometry (AAS), dilute 1000 mg/L of standard solutions (used for atomic absorption analysis) for each heavy metal with 0.5 mol/L nitric acid to produce a calibration curve. Correct it with standard test solution and measure optical density (or intensity). Inductively Coupled Plasma Spectrometer (ICP), or Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), can be used instead of Atomic Absorption Spectrometry (AAS) for measurement.

Mercury
Take 10 ~ 500 mg of specimen powder and test for lead using a mercury analyzer.

Heavy metals
Take 1 g (or 1 mL) of sample and test for heavy metals, following the third method for testing heavy metals. Add 3.0 mL of lead standard solution to matching liquid.

Determination
Follow appropriate relevant regulations for each drug and medication. In the absence of such regulations, carry out per following:
Plant derived medicines: below 5 ppm for lead; below 3 ppm for arsenic; below 0.2 ppm for mercury; and below 0.3 ppm for cadmium
Extract of herbal medicines: below 30 ppm for heavy metals
Medicines made only from herbs: below 30 ppm for heavy metals; below 5 ppm for lead; and below 3 ppm for arsenic.

Pesticide residues
Napropamide, p,p'-DDD, p,p'-DDE, o,p'-DDT & p,p'-DDT, Dieldrin, Myclobutanil, Methyl-pentachlorophenyl sulfide, Methoxychlor, a, ß, γ & d-BHC, Bifenthrin, Cypermethrin, Cyprodinil, Acetamiprid, Azoxystrobin, Aldrin, a, ß-Endosulfan & Endosulfan sulfate, Endrin, Chinomethionat, 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, Pyrimethanil, Hexaconazole, Fludioxonil.
Equipment: gas chromatography system [electron capture detector (ECD), nitrogen-phosphorous detector (NPD) and mass selective detector (MSD)]

Reagents and test solutions
(1) Solvents: solvents for testing agrochemical residues or equivalent
(2) Water: distilled water or equivalent
(3) Florisil: A cartridge (6mL capacity) of florisil (1 g)
(4) Filter agents: Celite® 545
(5) Undiluted standard solutions: dissolve standard specimen of each agrochemical into 100 ppm acetone.
(6) Standard solutions: mix and dilute each undiluted standard solution with acetone to appropriate level.
(7) Other reagents: reagents for testing agrochemical residues or special grade reagents.

Preparation of test solution
(1) Extraction: reduce 500 ~ 600 g of specimen to fine powder. Mix 5g of powder with water and let stand for 4 hours (the amount 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 vacuum using a vacuum pump, a conical flask and a Buchner funnel.
Transfer this solution into a 500 mL separating funnel and add 50 mL of a saturated solution of sodium chloride and 100 mL of distilled water. Add 70 mL of dichloromethane to the mixture and shake it vigorously; then allow for separation. Collect bottom layer (dichloromethane layer) in another separating funnel. After adding 70 mL of dichloromethane to the aqueous

layer, shake vigorously before allowing it separate and collecting the bottom layer (dichloromethane layer). Remove water from the dichloromethane layer by passing anhydrous sodium sulphate under vacuum and then dissolve it in 4 mL of hexane.

(2) Purification: pour 6 mL (1 g) of hexane into a florisil-charged cartridge (6 mL, 1 g) and elute it after 2 minutes. Pour 6 mL of hexane-acetone mixture (8:2) into the cartridge and re-elute. Then pour the extracted solution into the top end of the cartridge and slowly receive the elute after 2 minutes.

While the cartridge is still wet with the solvent, pour 5mL of hexane-dichloromethane-acetone mixture (50:48.5:1.5) for prochloraz and thifluzamid, hexane-acetone mixture (7:3) and collect the elute. Concentrate the elute under vacuum in a water-bath at below 40 °C and make a test solution by dissolving it in 2mL of acetone, hexane (1 → 5).

Conducting test

(1) Measurement conditions for gas chromatography

Electron capture detector (GC-ECD)

Column: A silica glass capillary column (inner diameter 0.25 mm, and length 30 cm) coated with 5 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness and a silica glass capillary column (inner diameter 0.25 mm, and length 30 m) coated with 50 % phenyl and 50 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Carrier gases and their quantity: Nitrogen, 1.0mL/min.

Column temperature: inject the specimen into the column at 80 ℃. After 2 minutes, increase temperature by 10 °C every minute until the temperature reaches 280 ℃, and let it stand for over 10 minutes (over 15 minutes if the column has been coated with phenyl 50 % and methyl silicone 50 %).

Injector temperature: 260 ℃, split mode (10:1)

Detector temperature: 280 ℃

Nitrogen-Phosphorous Detector (GC-NPD)

Column: A silica glass capillary column (inner diameter 0.25 mm, and length 30 cm) coated with 5 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness and a silica glass capillary column (inner diameter 0.25 mm, and length 30 m) coated with phenyl 50 % and methyl silicone 50 % (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Carrier gases and their quantity: Nitrogen, 1.0 mL/min.

Column temperature: inject the specimen into the column at 80 ℃. After 2 minutes, increase temperature by 10 °C every minute until the temperature reaches 280 ℃, and let it stand for 10 minutes (15 minutes if the column has been coated with phenyl 50 % and methyl silicone 50 %).

Injector temperature: 260 ℃, split mode (10:1)

Detector temperature: 280 ℃

Mass selective detector (GC-MSD)

Column: a silica glass capillary column (inner diameter 0.25 mm, and length 30 cm, used for mass selective detector) coated with 5 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Carrier gases and their quantity: Helium, 0.9mL/min.

Column temperature: inject specimen into the column at 100 °C. After 2 minutes, increase temperature by 10 °C every minute until temperature reaches 280 °C, and let it stand for over 15 minutes.

Injector temperature: 260 °C, split mode (10:1)

Interface temperature: 280 °C

Mobile phase flow rate: 1.0mL/min.

Qualitative test

(1) The retention time for each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the measurement conditions.

(2) Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are based on peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Metiram, Thiram and Propineb

Equipment: high performance liquid chromatography (UV-Detector)

Reagents and test solutions

(1) Solvents: solvents for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Undiluted standard solutions: dissolve standard thiram in methanol. Completely dissolve standard metiram and standard propineb in specimen extract solvent [mixture of 0.5g of L-Cysteine Hydrochloride Hydrate, and 100mL of 0.45 mol/L sodium hydroxide solution, 0.25 mol/L ethylenediaminetetraacetic acid, disodium salt (adjust the pH to 7.0 using hydrochloric acid)] to make 100 mg/L for immediate use.

(4) Standard solutions: dilute the undiluted standard solution with extracted solvent adjusted to the pH 7.0. Make sure that 1mL of the diluted solution undergo through processes of c) Preparation of test solution a) Extraction and b) Derivatization before diluting it to an appropriate concentration for use.

(5) Other reagents: for testing agrochemical residues, including methyl iodide, tetrabutylammonium hydrogen sulfate, ethylenediaminetetraacetic acid, disodium salt ethylenediaminetetraacetic acid, disodium salt, L-Cysteine Hydrochloride Hydrate, or special grade test solution

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to fine powder and place 20 g into a conical flask. Add 0.5 g of L-Cysteine Hydrochloride Hydrate to 80

mL of 0.45 mol/L sodium hydroxide solution (the pH: 9.5 ~ 9.6), containing 0.25 mol/L ethylenediaminetetraacetic acid. Immediately cover it with a stopper and shake it for 10 minutes. Filter this solution using a glass filter. Rinse the conical flask with 10 mL of the extracted solvent a few times, and then add this to the filtered solution.
After adding 5 mL of tetrabutylammonium hydrogen sulfate aqueous solution and 10 g of sodium chloride, shake it. Quickly adjust the pH to 7.0 using 2 mol/L hydrochloric acid, before transferring this solution to a 300 mL separating funnel.

※ Note: When grounding and homogenizing specimen, the agrochemicals that belong to the dithiocarbamate family dissolve quickly. As these agrochemicals are unstable in alkaline conditions, they start to dissolve immediately after extraction using the extracted solvent; the extraction must be carried out within 15 minutes to minimize rinsing and filtering time. The pH level should also adjust to 7.0.

(2) Derivatization: After putting 40 mL of dichloromethane-hexane mixture (1:1) containing 0.05 mol/L methyl iodide into the above separating funnel, shake it vigorously for 5 minutes and leave it to stand. After transferring the organic solvent layer (the top layer) to a 50 mL of centrifuge bottle and separating it for 5 minutes at 800rpm, mix 20 mL with 5 mL of dichloro methane solution (1 → 5) containing 1,2-propanediol and under vacuum extract solvents from which 1,2-propanediol has been removed under nitrogen flow. Immediately dissolve this in methanol to make a set amount of test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: A stainless steel column (inner diameter, 2 ~ 5 mm and length, 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 μm thickness.

Detectors: UV-Detector (measurement wavelengths of 272 nm)

Mobile phase: Water-acetonitrile-methanol mixture (65:22:13)

Flow rate: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.
Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test. Detections are made in the order of thiram, metiram and propineb.

Azocyclotin

Equipment: gas chromatography system [flame photometric detector (FPD)]

Reagents and test solutions

(1) Solvents: solvents for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: heat a florisil (60 ~ 100 mesh), used for column chromatography, at 130 °C overnight and then cool it in a desiccator.

(4) Filter agents: Celite® 545

(5) Undiluted standard solutions: dissolve standard azocyclotin in hexane to 100ppm.

(6) Standard solutions: dilute the undiluted standard solution with hexane to an appropriate concentration.

(7) Other reagents: reagents for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of specimen to fine powder, mix 30 g with 40mL of water and 10 mL of acetic acid. Add 100 mL of acetone to the mixture and blend for 3 minutes using a homogenizer before filtering it in a vacuum.
Rinse residue with acetone. Collect the filtered solution and transfer it to a separating funnel before extracting it twice using 100 mL of hexane each time. Remove water by passing anhydrous sodium sulphate through and concentrate the solution in a vacuum.

(2) Derivatization: After dissolving the concentrated solution in 20 mL of ether, add 3 mL of 3 mol/L magnesium chloride-tetrahydrofuran solution. Shake mixture and then let it stand for 10 minutes.
Hydrolyse by adding 10 mL of water, 1 mL of hydrochloric acid, before transferring it to a separating funnel. Rinse the flask with 10 mL ether and collect the solution in the separating funnel.
After discarding the aqueous layer (the bottom layer), and collecting the ether layer, remove water using anhydrous sodium sulphate, and concentrate to dry.

(3) Purification: Add hexane to 10 g of Florisil in a glass column (inner diameter, 20 mm and length, 30 mm). After dissolving concentrated solution in 10mL of hexane, transfer the solution to the column, elute it with 120mL of hexane and collect the elute. Concentrate and dry elute under a vacuum in a water-bath at below 40 °C and dissolve it in acetone to make set amount of test solution.

Conditions

(1) Measurement conditions for gas chromatography

Column: a silica glass capillary column (inner diameter 0.25 mm, and length 30 cm) coated with methyl silicone 50 %; and cyanoprophenyl 50 % (for gas chromatography) in 0.25 μm thickness, or equivalent.

Injector and detector temperature: 270 °C, 300 °C

Column temperature: inject specimen into column at 80 °C. After 2 minutes, increase temperature by 10 °C every minute until temperature reaches 260 °C, and let stand for over 20 minutes.

Carrier gases and their quantity: Nitrogen (1.0 mL/min.), hydrogen (3 mL/min.), air (30 mL/min.)

(2) Qualitative test

The retention time for each peak in the chromatogram of chemicals under study must correspond to peak in a standard solution, regardless of measurement conditions.

Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in qualitative test.

Carbendazim

Equipment: high-speed liquid chromatography system [UV-Detector or Fluorescence Detector]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: heat florisil used for column chromatography at 130 °C overnight and cool in a desiccator.

(4) Undiluted standard solutions: dissolve standard carbendazim to 100 ppm methanol.

(5) Standard solutions: dilute, undiluted standard solution with methanol to an appropriate concentration.

(6) Other reagents: For testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 10 g with 4 g of sodium L-ascorbate, 40 mL of water, 80 mL of methanol and 5 g of hyflosuper cell. Shake the mixture for 1 hour before filtering it under vacuum. Rinse the container using 50 mL of methanol to collect the residues and add the residues to filtered solution.

(2) Extraction (2nd time): transfer the extracted solution to a separating funnel and add 200 mL of distilled water, 20 mL of a saturated solution of sodium chloride before adjusting 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 aqueous layer to 6 ~ 7, extract the solution from 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 in a water-bath at below 40 °C.

(3) Purification: add hexane to 5 g of Florisil in a glass column (inner diameter 15 mm, and length 300 mm). After mixing agrochemical residues with 5 mL of hexane-acetone mixture (7:3), elute it.

Concentrate and dry the elute in a water-bath at below 40 °C, and dissolve it in 2 mL of methanol to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: A stainless steel column (inner diameter, 2 ~ 5 mm and length, 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 μm thickness.

Column temperature: 40 °C

Mobile phase: 0.01 mol/L potassium phosphate TS-methanol mixture (6:4)

Detectors: UV-Detector (measurement wavelengths of 285 nm), fluoro spectrophotometer (excitation wavelengths 280 nm, fluorescence wavelengths 315 nm)

Flow rate: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to peak obtained in a standard solution regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Difenoconazole

Equipment: gas chromatography system [Nitrogen-Phosphorous Detector (NPD)]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: a cartridge (capacity of 6 mL) filled with florisil (1 g) stationary phase

(4) Filter agents: Celite® 545

(5) Undiluted Standard solutions: dissolve standard difenoconazole in acetone to 100 ppm.

(6) Standard solutions: dilute undiluted standard solution with acetone to an appropriate concentration.

(7) Other reagents: For testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 50 g with 100 mL of acetone, before shaking it for 30 minutes. Filter the extracted solution using Celite® 545 under vacuum and add 50 mL of a saturated solution of sodium chloride before extracting the solution twice using 50 mL of hexane each time.

Passing anhydrous sodium sulphate through the hexane

layer to remove water and concentrate it under vacuum in a water-bath at below 40 °C before dissolving it in 5mL of hexane. Remove water by passing anhydrous sodium sulphate through hexane layer and concentrate it in a water-bath at below 40 °C before dissolving it in 5mL of hexane.

(2) Purification: after eluting 5 mL hexane into the florisil-charged cartridge at a speed of 2 ~ 3 drops per second, make sure that the cartridge absorbs the extracted solution. Elute 20 mL of hexane-acetone mixture (95:5). Elute 40 mL of hexane-acetone mixture (7:3). Concentrate the elute under vacuum in a water-bath at below 40 °C and make a test solution by dissolving it in 2mL of acetone.

Conducting test

(1) Measurement conditions for gas chromatography

Column: a silica glass capillary column (inner diameter 0.25 mm, and length 30 cm) coated with phenyl 50 % and methyl silicone 50 % (in use for gas chromatography) in 0.25 µm thickness, or equivalent.

Test solution injector and detector temperature: 280 °C

Column temperature: inject the specimen into the column at 100 °C. After 1 minute, increase temperature by 10 °C every minute until the temperature reaches 250 °C, and let it stand for over 12 minutes.

Carrier gases and their quantity: Nitrogen, 1.0mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of measurement conditions.

Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Imidacloprid

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Filter agents: Celite® 545

(4) Silica gel cartridges: cartridge (capacity of 6 mL) filled with silica gel (1 g) stationary phase (for SPE) or equivalent.

(5) Undiluted standard solutions: dissolve standard imidacloprid in water-acetonitrile mixture (8:2) to 100 ppm.

(6) Standard solutions: Dilute the undiluted standard solution with water-acetonitrile mixture (8:2) to an appropriate concentration.

(7) Other reagents: for testing agrochemical residues or special grade reagents.

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 25 g with 100 mL of acetonitrile and 100 mL of water. Homogenize the mixture for 5 minutes at a high speed.

After filtering the extracted solution under vacuum and concentrate 100 mL of the filtered solution under vacuum in a water-bath at below 40 °C until all that is left is water.

After extracting the solution from the aqueous layer twice using 50 mL of cyclohexane each time, discard the cyclohexane layer and extract the solution twice using 50 mL of dichloromethane each time.

Pass anhydrous sodium sulphate through the extract solution to remove water and concentrate it under vacuum in a water-bath at below 40 °C before dissolving it in 2 mL of dichloromethane.

(2) Purification: after making sure that previously activated silica gel cartridge absorbs the extract solution, elute with 10 mL of hexane-ethyl acetate mixture (1:1) and then elute with 15 mL of ethyl acetate-hexane mixture (7:3).

Concentrate and dry the solution before dissolving it in an acetonitrile-water mixture (1:1) and making a set amount of test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter, 2 ~ 5 mm and length, 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 µm thickness.

Detectors: UV-Detector (measurement wavelengths of 270 nm)

Mobile phase 0.01 mol/L disodium hydrogen phosphate (the pH 6.5) acetonitrile mixture (75:25)

Flow rate: 0.8 mL/min.

Column temperature: 40 °C

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Iminoctadine

Equipment: high-speed liquid chromatography system (Fluorescence Detector)

Reagents and test solutions

(1) Solvents: solvents for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Undiluted Standard solutions: dissolve standard iminoctadine triacetate in water and make100 ppm iminoctadine (Iminoctadine scale factor: 0.664).

(4) Standard solutions: dilute undiluted standard solution with water to an appropriate concentration.

(5) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 7g of Guanidine hydrochloride. Add 100 mL of methanol to the solution and homogenize it for 5 minutes at a high speed.

After filtering the extracted solution under vacuum and adding 50 mL of 2 mol/L sodium hydroxide test solution, 50 mL of water and 100 mL of chloroform to the filtered solution, collect the organic solvent layers for 2 minutes in 3 stages. Add 2 mL of 1 mol/L sulfuric acid test solution and 40mL of water, and collect the aqueous layers in stages. Add 0.5 mL of 1 mol/L sulfuric acid test solution and 20 mL of water, and collect the aqueous layers in stages.

After mixing the aqueous layers and concentrating it in a water-bath at about 70 °C to 2 mL, add 5 mL of sodium acetate aqueous solution (16 → 100) to the concentrated solution to make a test solution.

Conducting test

Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter 2 ~ 5 mm, and length 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 μm thickness.

Detectors: fluoro spectrophotometer (excitation wavelengths 305 nm, fluorescence wavelengths 500 nm)

Mobile phase:

A: Water-liquid ammonia (28) mixture (69:1) (Adjust its pH to pH 2.5 using 60 % perchloric acid.)

B: Diluted methanol (2 → 5)

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).

Column temperature: 40 °C

Flow rate: 0.7 mL/min.

Post reaction pump flow rate: 0.7 mL/min. (0.5 mol/L sodium hydroxide solution, 0.7 mL/min and ninhydrin solution (3 → 20), 0.7 mL/min.) (or other optimum conditions)

Reactor temperature: 80 °C

Qualitative test

(1) The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

(2) Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Pymetrozine

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Cartridge: a cartridge (capacity of 6mL) filled with 250mg of graphitized carbon

(4) Filter agents: Celite® 545

(5) Undiluted Standard solutions: dissolve standard pymetrozine in acetonitrile to 500ppm.

(6) Standard solutions: dilute undiluted standard solution with acetonitrile to an appropriate concentration.

(7) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 100 mL of methanol and 30 mL of water. Leave the mixture at room temperature for 2 hours and then extract it by shaking for 1 hour.

After filtering the extracted solution under vacuum using Celite® 545, rinse off the residues with 100 mL of methanol and mix it with the filtered solution to make exactly 250 mL.

Transfer 100 mL of the solution to a separating funnel and add 100 mL of hexane. Shake the mixture and then discard the hexane layer. Collect the methanol layer and concentrate it under vacuum to 5 mL.

(2) Purification: after making sure that previously activated graphitized carbon-charged cartridge column (250 mg, 6 mL) absorbs the extract solution, elute with 5 mL of methanol water mixture (1:1), 5 mL of methanol acetonitrile mixture (7:3) and 5mL of ethyl acetate to remove foreign matter.

Dry it under vacuum for 3 minutes and elute with 30mL of dichloromethane. Collect the elute.

After concentrating and drying the elute under vacuum

in water-bath at below 35 ℃, make a test solution by dissolving it in 2mL of acetonitrile.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter, 2 ~ 5 mm and length, 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 μm thickness.

Detectors: UV-Detector (measurement wavelengths of 300 nm)

Mobile phase: Water-acetonitrile mixture (87 : 13)

Flow rate: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Thiamethoxam

Equipment: high-speed liquid chromatography system [UV-Detector]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Silica cartridge: A cartridge (capacity of 6 mL) filled with silica gel (1 g)

(4) Filter agents: Celite® 545

(5) Undiluted Standard solutions: dissolve standard thiamethoxam in acetonitrile to 100 ppm.

(6) Standard solutions: dilute undiluted standard solution with acetonitrile to an appropriate concentration.

(7) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 100 mL of ethyl acetate-acetone mixture (3:2). Shake the mixture for 30 minutes.

Pass the extracted solution through Celite® 545 and remove the solvent by concentrating it under vacuum. Add 50 mL of sodium chloride test solution and extract the solution twice by adding 50 mL of dichloromethane each time.

Remove water from the extracted solution by passing anhydrous sodium sulphate through the solution. After concentrating and drying under vacuum, dissolve it in 5mL of hexane-acetone mixture (9:1).

(2) Purification: after making sure that previously silica cartridge absorbs the extract solution, elute with 10 mL of hexane-acetone mixture (9:1) and then elute with 20 mL hexane-acetone mixture (3:2). Collect the elute.

Concentrate and dry the elute under vacuum before dissolving it in 2 mL of acetonitrile to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column A stainless steel column (inner diameter 2 ~ 5 mm, and length 20 ~ 30 cm) coated with octadecylsilica gel (in use for liquid chromatography) in 5 μm thickness.

Detectors: UV-Detector (measurement wavelengths of 254 nm)

Mobile phase: acetonitrile·water mixture (1:1)

Flow rate: 1.0 mL/min.

Column temperature: 25 °C

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Triforine

Equipment: gas chromatography system [electron capture detector (ECD)]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Silica gel: silica gel (70 - 230 mesh) for column chromatography

(4) Undiluted Standard solutions: dissolve standard triforine in acetone to 100 ppm

(5) Standard solutions: dilute undiluted standard solution with acetone to an appropriate concentration.

(6) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 200 mL of acetone. Homogenize and filter the mixture under vacuum.

After removing acetone and transferring it to a separating funnel, add 200 mL of sodium chloride solution (1 → 20) and extract the solution twice using 100 mL of benzene each time.

Collect the benzene layer and concentrate it under vac-

uum before dissolving it in 5 mL of acetone.

(2) Purification: elute a glass column (inner diameter, 10 mm and length, 40 mm), filled with 5 g of filled with 2 g of anhydrous sodium sulphate and 5 g of silica gel that has been activated at 130 °C overnight, with 50 mL of hexane.

Dissolve the concentrated solution in a small amount of benzene and make sure that the column absorbs it. After eluting with 150 mL of hexane acetone mixture (9:1) and eluting with 100 mL of hexane acetone mixture (7:3) again, collect the elute. Concentrate and dry the elute under vacuum before dissolving it in methanol-ethyl acetate mixture (1:1) to make a set amount of a test solution.

Conducting test

(1) Measurement conditions for gas chromatography

Column: a silica glass capillary column (inner diameter, 0.25 mm and length, 30 cm) coated with phenyl 50 % and methyl silicone 50 % (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Test solution injector temperature: 270 ℃

Detector temperature: 280 ℃

Column temperature: 150 ℃

Carrier gases and their quantity: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the measurement condition.

Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Dithianon

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Silica gel: for column chromatography (60 ~ 100 mesh)

(4) Undiluted Standard solutions: dissolve standard dithianon in an acetone solution containing acetic acid (1 → 20) to 100 ppm.

(5) Standard solutions: dilute undiluted standard solution with an acetone solution containing acetic acid (1 → 20) to an appropriate concentration.

(6) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 5 mL of 4 mol/L hydrochloric acid 100 mL of acetone. Shake the mixture for 30 minutes. Filter the extracted solution under vacuum using Celite® 545, a vacuum pump, a conical flask and a Buchner funnel.

After removing acetone by concentrating the extracted solution under vacuum in a water-bath at below 40 °C, transfer it to a separating funnel by using 50 mL of sodium chloride solution (1 → 20) and 50 mL of hexane. Shake the solution vigorously and allow it to separate.

After transferring the bottom layer (aqueouslayer) to another separating funnel, add 50 mL of hexane. Shake the solution vigorously and allow it to separate. Collect the hexane layer and remove water by passing anhydrous sodium sulphate through the layer. Concentrate it under vacuum and then dissolve it in 5 mL of dichloromethane.

(2) Purification: after eluting a glass column (inner diameter, 14mm and length, 400 mm), filled with 5g of silica gel (60 ~ 100 mesh) and 2g of anhydrous sodium, with 50 mL of hexane, make sure that 5mL of the extracted solution is absorbed. After eluting with 50 mL of benzene and concentrating the elute under vacuum, dissolve it in 1mL of acetonitrile to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter, 4.6 mm and length, 25 cm) coated with octadecyl-silica gel (in use for liquid chromatography) in 5 μm thickness.

Column temperature: room temperature (30 °C)

Mobile phase methanol·water·acetic acid mixture (70:30:1)

Detectors: UV-detector (measurement wavelengths of 254 nm)

Flow rate: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Fenpyroximate

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: heat a florisil, used for column chromatography, at 130 °C overnight and then cool it in a desiccator.

(4) Undiluted Standard solutions: dissolve standard fenpyroximate in acetone to 100 ppm.

(5) Standard solutions: dilute undiluted standard solution with acetone to an appropriate concentration.

(6) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 100mL of acetone (7 → 10). Shake the mixture for 30 minutes. Filter the extracted solution under vacuum using Celite® 545, a vacuum pump, a conical flask and a Buchner funnel.
After removing acetone by concentrating the extracted solution under vacuum in a water-bath at below 40 °C, transfer it to a separating funnel by using 50 mL of sodium chloride solution (1 → 20) and 50 mL of hexane. Shake the solution vigorously and allow it to separate.
After transferring the bottom layer (aqueous layer) to another separating funnel, add 50 mL of hexane. Shake the solution vigorously and allow it to separate. Collect the hexane layer and remove water by passing anhydrous sodium sulphate through the layer. Concentrate it under vacuum and then dissolve it in 5 mL of dichloromethane.

(2) Purification: Place 30 mL of dichloromethane in a glass column (inner diameter, 14 mm and length, 400 mm), filled with 5 g of florisil and 2 g of anhydrous sodium sulphate. Discard the elute and add 5 mL of the extracted solution to the column making sure that the solution is absorbed.
Elute it with 150 mL of dichloromethane-ethyl acetate mixture (98: 2). Collect the elute and concentrate it under vacuum before dissolving it in 1mL of acetonitrile to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography
Column: a stainless steel column (inner diameter, 4.6 mm and length, 25 cm) coated with octadecyl-silica gel (in use for liquid chromatography) in 5 μm thickness.
Column temperature: room temperature (30 °C)
Mobile phase acetonitrile·water mixture (9:1)
Detectors: UV-detector (measurement wavelengths of 254 nm)
Flow rate: 1.0 mL/min.

(2) Qualitative test
The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.
Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Sethoxydim

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Silica cartridge: a cartridge (capacity of 6 mL) filled with silica gel (500 mg)

(4) Undiluted Standard solutions: dissolve standard sethoxydim in methanol to 100 ppm.

(5) Standard solutions: dilute undiluted standard solution with methanol to an appropriate concentration.

(6) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 10 g with 100 mL of methanol (4 → 5). After shaking the mixture vigorously for I hour, filter the extracted solution under vacuum using a vacuum pump, a conical flask and a Buchner funnel.
After removing methanol by concentrating the extracted solution under vacuum, transfer it to a separating funnel and add 50 mL of sodium chloride and 200 mL of water. Add 70 mL of dichloromethane and shake the solution vigorously before allowing it to separate.
After transferring the bottom layer (dichloromethane layer) to another separating funnel, add 50 mL of dichloromethane to the aqueous layer again. After shaking the solution vigorously and allowing it to separate, collect the dichloromethane layer. Remove water by passing anhydrous sodium sulphate through the dichloromethane layer. Concentrate it under vacuum and then dissolve it in 5mL of hexane-ethyl acetate mixture (95:5).

(2) Purification: Place 6mL of hexane into the silica cartridge (6mL, 500 mg) and elute it after 2 minutes. Elute the cartridge with 6mL of hexane-ethyl acetate mixture (95:5) in the same way.
Place the extracted solution into the column in the top end of the cartridge and slowly elute it after 2 minutes. While the cartridge is wet with the solvent, elute it with 5mL of hexane-acetone mixture (9:1) and collect the elute. Concentrate the elute under vacuum in a water-bath at below 40 °C and dissolve it in 2 mL of methanol to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter, 4.6 mm and length, 25 cm) coated with octadecyl-silica gel (in use for liquid chromatography) in 5 μm thick-

ness.

Mobile phase methanol water mixture (7:1)

Detectors: UV-detector (measurement wavelengths of 280 nm)

Flow rate: 1.0mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Fluazifop-butyl

Equipment: high-speed liquid chromatography system (UV-Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: for column chromatography

(4) Undiluted Standard solutions: dissolve standard flauzifop-P-butyl in acetonitrile to 100ppm.

(5) Standard solutions: dilute undiluted standard solution with acetonitrile to an appropriate concentration.

(6) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 10 g with 100mL of acetonitrile. Homogenize it for about 3 minutes at a high speed. Filter the extracted solution under vacuum using Celite® 545 and a Buchner funnel.

Transfer the filtered solution to a separating funnel that contains 100 mL of a saturated solution of sodium chloride and 400 mL of water. Add 50 mL of dichloromethane, and shake the solution vigorously before allowing it to separate.

After transferring the bottom layer (dichloromethane layer) to another separating funnel, add 50 mL of dichloromethane to the aqueous layer again. After shaking the solution vigorously and allowing it to separate, collect the dichloromethane layer.

Remove water by passing anhydrous sodium sulphate through the dichloromethane layer. Concentrate it under vacuum and then dissolve it in 5 mL of dichloromethane-n-hexane-acetonitrile mixture (50: 49.65:0.35).

(2) Purification: place hexane in a glass column (inner diameter, 20 mm and length, 30 mm), filled with 10g of florisil and 2 g of anhydrous sodium sulphate. Rinse the column with 50 mL of hexane. After making sure that the column absorbs the extracted solution, elute it with 70 mL of dichloromethane·n-hexane·acetonitrile mixture (50 : 48.5 : 1.5). Collect the elute. Concentrate the elute under vacuum in a water-bath at below 40 °C and dissolve it in 2 mL of acetonitrile to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography

Column: a stainless steel column (inner diameter,4.6 mm and length, 25 cm) coated with octadecyl-silica gel (in use for liquid chromatography) in 5 μm thickness.

Column temperature: 40 °C

Mobile phase: Acetonitrile-diluted formic acid (1 → 1000) mixture (7:3)

Detectors: UV-detector (measurement wavelengths of 225 nm)

Flow rate: 1.0 mL/min.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Oxolinic acid

Equipment: high-speed liquid chromatography system (UV-Detector or Fluorescence Detector)

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Undiluted Standard solutions: dissolve standard oxolinic acid in methanol-0.25 mol/L sodium hydroxide solution mixture (9:1) to 100 ppm.

(4) Standard solutions: dilute undiluted standard solution with methanol to an appropriate concentration.

(5) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 10 g with 30 mL of water and 5 mL of 5 mol/L hydrochloric acid before leaving it for 30 minutes. After adding 100 mL of acetone and homogenizing it for about 5 minutes at a high speed, filter it under vacuum. Transfer the filtered solution to a separating funnel that contains 50 mL of a saturated solution of sodium chloride and 450 mL of water.

Add 100 mL of hexane and shake the solution vigorously before allowing it to separate. Discard the hexane layer and add 70 mL of dichloromethane to the aqueous layer. Shake the solution vigorously and allow it to

separate.
Transfer the lower layer (dichloromethane) to another separating funnel. Add 70 mL of dichloromethane to the water layer, shake it vigorously and then allow it to separate. Concentrate it under vacuum and then dissolve it in 10 mL of 0.01 mol/L oxalic acid-acetonitrile-methanol mixture (6:3:1) to make a test solution.

Conducting test

(1) Measurement conditions for high-speed liquid chromatography
Column: a stainless steel column (inner diameter, 4.6 mm and length, 25 cm) coated with octadecyl-silica gel (in use for liquid chromatography) in 5 μm thickness.
Column temperature: 40 °C
Mobile phase: diluted acetic acid (1 → 200)-acetonitrile-methanol mixture (7:3:1)
Detectors: Fluoro spectrophotometer (excitation wavelengths 330 nm, fluorescence wavelengths 365 nm) or UV-detector (measurement wavelengths of 360 nm)
Flow rate: 0.8 mL/min.

(2) Qualitative test
The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.
Using a LC/MS/MS, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Pencycuron

Equipment: gas chromatography system [nitrogen-phosphorous detector (NPD)]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: Heat a florisil (60 ~ 100 mesh), used for column chromatography, at 130 °C overnight and then cool it in a desiccator.

(4) Filter agents: Celite® 545 (celite 545)

(5) Undiluted Standard solutions: dissolve standard pencycuron in acetone to 100 ppm.

(6) Standard solutions: dilute the undiluted standard solution with acetone to an appropriate concentration.

(7) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 500 ~ 600 g of the specimen to a fine powder and mix 20 g with 80 mL of acetone. After homogenizing for 5 minutes, filter it under vacuum using a vacuum pump, a conical flask and a Buchner funnel.
Transfer the filtered solution to a separating funnel with a capacity of 500 mL and add 50 mL of a saturated solution of sodium chloride and 450 mL of water.
After transferring the bottom layer (dichloromethane layer) to another separating funnel, add 60 mL of dichloromethane to the aqueous layer again. After shaking the solution vigorously and allowing it to separate, collect the dichloromethane layer. Remove water by passing anhydrous sodium sulphate through the dichloromethane layer. Concentrate it under vacuum and then dissolve it in 10mL of hexane.

(2) Purification: place hexane in a glass column (inner diameter, 20 mm and length, 30 mm), filled with 10g of florisil. Elute the column with 50 mL of hexane and pour the concentrated solution into the column. After eluting it with 50 mL of hexane solution containing acetone (1 → 20) and elute it with 60 mL of hexane solution containing acetone (1 → 20) again and collect the elute. Concentrate the elute under vacuum in a water-bath at below 40 °C and dissolve the residues in 1mL of acetone.

(3) Derivatization: after adding 0.5mL of dimethyl sulfoxide to the above solution, add about 0.2g of sodium hydride and 0.5 mL of methyl iodide. Cover it with a stopper and let it stand for 30 minutes at 30 °C, shaking it occasionally. Add 5 mL of hexane and shake it vigorously for 1 minute before slowly adding 10 mL of distilled water in drops. When it stops generating hydrogen gas, transfer it to a separating funnel using a small amount of hexane and distilled water, and shake it vigorously for 5 minutes.
After removing water by passing the hexane layer through the anhydrous sodium sulphate column and rinsing the column with about 20 mL of hexane, concentrate the solution under vacuum in a water-bath at below 40 °C and dissolve the residues in hexane to make a set amount of a test solution.

Conducting test

(1) Measurement conditions for gas chromatography

Column: a silica glass capillary column (inner diameter, 0.25 mm and length, 30 cm) coated with 5 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness and a silica glass capillary column (inner diameter, 0.25 mm and length, 30 m) coated with phenyl 50 % and methyl silicone 50 % (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Carrier gases and their quantity: Nitrogen, 1.0mL/min.

Column temperature: 230 °C

Injector temperature: 250 °C, split mode (10:1)

Detector temperature: 280 °C

(2) Qualitative test
The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

(3) Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Methidathion, Triazothe pHos, Fenitrothion, Phenthoate

Equipment: gas chromatography system [flame photometric detector (FPD) or nitrogen-phosphorous detector (NPD)]

Reagents and test solutions

(1) Solvents: for testing agrochemical residues or equivalent

(2) Water: distilled water or equivalent

(3) Florisil: Heat a florisil (60 ~ 100 mesh), used for column chromatography, at 130 °C overnight and then cool it in a desiccator.

(4) Filter agents: Celite® 545 (celite 545)

(5) Undiluted Standard solutions: dissolve standard relevant agrochemicals in acetone to 100 ppm.

(6) Standard solutions: dilute undiluted standard solution with acetone to an appropriate concentration.

(7) Other reagents: for testing agrochemical residues or special grade reagents

Preparation of test solution

(1) Extraction: reduce 20 ~ 30 g of specimen to a fine powder and mix 2 g with 20 mL of distilled water before leaving it for 1 hour. Add 100 mL of acetone to the solution and blend it for 3 minutes using a homogenizer before filtering it under vacuum. Rinse off the residues with a small amount of acetone.

After collecting the filtered solution and concentrating it under vacuum to 50 mL, transfer it to a separating funnel. Add 500 mL of distilled water and 50 mL of a saturated solution of sodium chloride before extracting the solution twice using 50 mL of dichloromethane. Remove water by passing anhydrous sodium sulphate and then concentrate the solution under vacuum.

(2) Purification: add hexane to a glass column (inner diameter, 20 mm and length, 30 mm), filled with 10 g of florisil and 2 g of anhydrous sodium sulphate. After dissolving the concentrated solution in 10mL of hexane and transferring the solution to the column, elute it with 30 mL of hexane ethyl acetate mixture (98 : 2) and discard the elute. Elute it with 60mL of hexane ethyl acetate mixture (8 : 2) again and collect the elute. Concentrate and dry the elute under vacuum in a water-bath at below 40 °C and dissolve it in acetone to make a set amount of a test solution.

Conducting test

(1) Measurement conditions for gas chromatography

Column: a silica glass capillary column (inner diameter, 0.25 mm and length, 30 cm) coated with 5 % phenyl and 95 % methyl silicone (in use for gas chromatography) in 0.25 μm thickness, or equivalent.

Injector and detector temperature: 220 °C, 260 °C (adjust it if necessary)

Column temperature: Inject the specimen at 150 °C and increase temperature by 5 °C every minute until temperature reaches 260 °C. Maintain this temperature for over 10 minutes (adjust it if necessary)

Carrier gases and their quantity: Adjust the amount of nitrogen and helium if necessary.

Gas flow rate in detectors: Adjust the amount of hydrogen and air if necessary.

(2) Qualitative test

The retention time of each peak in the chromatogram of the chemicals under study must correspond to the peak obtained in a standard solution, regardless of the conditions of measurement.

Using a GC-MSD detector, components of each agrochemical can be identified by retention time and mass spectrum.

Quantitative test: Evaluations are made using the peak heights and peak areas, obtained under the same conditions as in the qualitative test.

Determination

Follow the standards stated in the clauses of the relevant regulations for each drug and medication. In the absence of specified regulations, the limit for herbal medicines and herbal extracts is as follows: Total amount of DDT (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. (Test can be exempted for the herbal extracts whose raw material has been tested)

If agrochemicals that are not listed in Clause1 have been detected, the following items are applicable for determination.

(1) Assessment in accordance with Clause “Pesticide Residues” in the European Pharmacopoeia.

(2) When detecting agrochemicals that are not listed in the European Pharmacopoeia, the Head of the Korea Food & Drug Administration can determine safety using the following method.

$$\frac{ADI \times M}{MDD \times 100}$$

ADI: Permitted daily exposure to the relevant agrochemicals (mg/kg/day)

M: Average adult weight (60 kg)

MDD: Daily dosage of the relevant herbal medicines (kg)

Despite Clause 2, the following cases follow special standards in determining safety.

(1) If a herbal medicine item contains agrochemical residues other than those that meet the guidelines

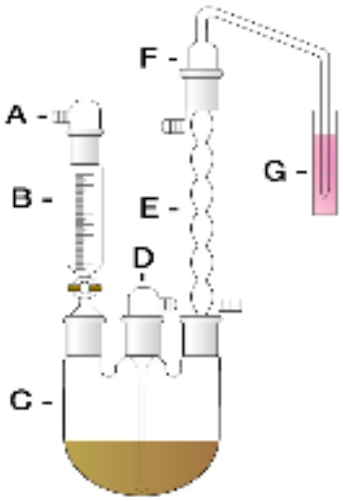
for permitted residue levels of agrochemicals (announced by Korea Food & Drug Administration), then follow the "Standards for Agrochemical Residues in Agricultural Products" and the "Provisional Standards for Agrochemical Residues in Processed Food" in the "Food Standard and Requirement" (announced by Korea Food & Drug Administration).

(2) If 'dried orange peel' and 'natsudaidai peel' are found to contain agrochemical residues that are listed as permitted agrochemical residues for tangerines in Standards and Specfications of Foods (announced by Korea Food & Drug Administration), the standards will be determined by multiplying the level for 'tangerines' by each processing factor (dried orange peel, 8 and natsudaidai peel, 14)

Sulfur dioxide

1) Equipment

Use the equipment in the drawing below.



A: hose connection
B: separating funnel (capacity of 100 mL or more)
C: distilling flask (1,000 mL)
D: gas injection tube
E: allihn condenser (300 mm)
F: bubbler
G: collector (inner diameter, 25 mm and depth, 150 mm)

Test solution

Methyl Red test solution: Dissolve 250 mg of Methyl Red in ethanol to make 100 mL.

3 % hydrogen peroxide solution: Add water to 10 mL of hydrogen peroxide (30) to make 100 mL, and add 3 drops of Methyl Red before adding 0.01 mol/L sodium hydroxide solution to make a light yellow solution (make it just before using it).

Methods

Pour 400 mL of water into a distilling flask (C). Place a cork in the separating funnel (B) and add 90 mL of 4 mol/L hydrochloric acid. Pass nitrogen gas through the gas injection tube (D) at the speed of 0.21 L/min. and pass cold water through the Allihn condenser (E). Pour 30 mL of 3 % hydrogen peroxide solution into the collector (G). Disconnect the separating funnel (B) after 15 minutes. Mix 50g of the specimen powder and 100mL of water-ethanol mixture (95 : 5), and put the mixture into the distilling flask (C). After reconnecting the separating funnel (B), open the cork and pour 4 mol/L hydrochloric acid into the distilling flask (C), leaving 2 ~ 3 mL in the separating funnel. Place the cork and heat the distilling flask. After boiling the mixture in the distilling flask for 1 hour and 45 minutes, 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 until the solution turns and remains yellow for more than 20 seconds (V_1 mL). Repeat the same process to carry out a (V_2 mL).

※ 1 mL of 0.01 mol/L sodium hydroxide solution = 320 μg SO2

$$\text{sulfurdiox ide}(\frac{\text{mg}}{\text{kg}}) = \frac{320 \times (V_1 - V_2) \times f}{S}$$

V_1: consumption of 0.01 mol/L sodium hydroxide solution (mL)

V_2: consumption of 0.01 mol/L sodium hydroxide solution (mL) during a blank test

f : titer of 0.01 mol/L sodium hydroxide solution

S: the amount of used specimen (g)

Mycotoxin

Preparation of test solution

Reduce 500 ~ 600 g of the specimen to a fine powder and mix 5.0 g with 100 mL of diluted methanol (7 → 10). After extracting by sonication for 30 minutes and filtering it, add diluted methanol (7 → 10) to make a 100 mL solution. Pippette 10 mL of this solution and add water up to 80mL to make an 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 the speed of 3 mL/min. Discard the elute. Pass weak vacuum through immunoaffinity column (for aflatoxin) for 5 ~ 10 seconds or pass air for 10 seconds using a syringe to dry it. Pour 0.5 mL of methanol through the dried immunoaffinity column (for aflatoxin) to remove the elute using gravity. Let it stand for 1 minute and then pass 0.5 mL of methanol twice. Add all the elute and make it 5 mL by adding water. Make sure that the flow rate does not exceed 5mL/min. If the elute is clear, use it as a test solution, and filter it using a 0.45 μm filter if necessary.

Preparation of standard solutions

Mix 1.0 mg of each standard aflatoxin B_1, B_2, G_1 and G_2 with toluene·acetonitrile mixture (98 : 2) to make 100mL to make and use this as a first undiluted standard solution of aflatoxin mixture. Mix 1mL of this first undiluted standard solution with toluene·acetonitrile

mixture (98 : 2) to make 100 mL and use this as a second undiluted standard solution.

Making of calibration curve
Dilute the above undiluted standard solution to an appropriate concentration to make a standard solution and a calibration curve. If the concentration of the test solution is outside the range of the calibration curve, adjust the concentration to within the range of the calibration curve.

Methods
Use 10 ~ 500 μL of the test solution and standard solution to test in accordance with the liquid chromatography. 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 (B_1, B_2, G_1, G_2).

$$\text{The amount of aflatoxin B1 in the specimen} = \frac{V_1 \times V_2}{m} \times \frac{(C_{B1} + C_{B2} + C_{G1} + C_{G2})}{V_i}$$

m = Mass of the dried specimen taken for analysis(g)
V_1 = Volume of the solvent used for extraction (mL)s
V_i = Volume of the elute solution used for aliquot taken for immunoaffinity clean-up (mL)
V_2 = Final volume of solution after elution from the immunuaffinity column and dilution (mL)
C_{B1} = Concentration of aflatoxin B_1, measured in the test solution (ng/mL)
C_{B2} = Concentration of aflatoxin B_2, measured in the test solution (ng/mL)
C_{G1} = Concentration of aflatoxin G_1, measured in the test solution (ng/mL)
C_{G2} = Concentration of aflatoxin G_2, measured in the test solution (ng/mL)

<Conditions>
Detectors: fluorophotometer (excitation wavelength 365 nm, emission wavelength 435 nm)
Column: A stainless steel column (inner diameter, 4.6 mm and length, 15 ~ 25 cm) coated with octadecylsilyl-silica gel (in use for liquid chromatography) in 3 ~ 5 μm thickness, or equivalent.
Mobile phase: A: Water-methanol·acetonitrile mixture (6: 3: 2 *V/V/V*)
B: Add 0.12 g of potassium bromide and 350 μL of diluted nitric acid per liter of mobile phase A
Flow rate: 1.0 mL/min.

Post-column derivatization equipment: Any of the following three can be selected for the test.
Post-column derivatization with pyridinium hydrobromide perbromide (PBPB)

Pulseless pumps
Polytetrafluoroethylene reaction tube : 0.45 m × 0.5 mm
Mobile phase A
Derivatization reagent: Dissolve 50 mg of para-bromophenacyl bromide in 1000 mL of water (store protected from light and use within 4 days)
Flow rate of the derivatization reagent: 0.4 mL/min.

Photochemical reactor (PHRED)
(1) 254 nm low pressure mercury UV lamps (minimum 8 W)
(2) Polished support plate
(3) Knitted reactor coil: Polytetrafluoroethylene tubing around the UV bulb
(4) Exposure time : 2 min.
(5) Mobile phase A

Electrochemically generated bromine (KOBRA)
(1) KOBRA cell: an electrochemical cell that generates a reactive form of bromine for derivatization of aflatoxins, resulting in enhanaced fluorescence
(2) Direct-current supply in series with the KOBRA-cell: providing a constant current of about 100 uA.
(3) Polytetrafluoroethylene reaction tube : 0.12 m × 0.25 mm
(4) Mobile phase B

※ Note) After purchasing and storing a standard product over a certain period of time, the concentration must be corrected by carrying out the following procedures before use.

※ Add toluene·acetonitrile mixture (98 : 2) to 1.0mg of standard aflatoxin B1 to make a 100mL solution, and use this as the first undiluted standard aflatoxin B1 solution. Use this undiluted standard solution to measure optical density (between 330 ~ 370 nm) in accordance with the UV Spectrophotometer, used as a general test method by Korean Pharmacopeia, and calculate the concentration (μg/mL) of the first undiluted aflatoxin B1 standard solution using the following formula.
Concentration (μg/mL) of the first undiluted aflatoxin B1 ($C_{17}H_{12}O_6$) standard solution

$$\frac{A \times M \times 100}{\varepsilon \times \zeta}$$

A = absorbance determined at the maximum of the absorption curve
M = molar mass of aflatoxin B1: 312 g /mol
e = molar absorptivity of aflatoxin B1 in the toluene·acetonitrile mixture: 1930 m2/mol
ζ = optical path length of the cell, used to measure optical density: 1 cm

Note) Store the undiluted standard solution protected from light and below 4 °C.
Do not remove the aluminum foil until the contents

have reached room temperature.

Benzopyrene
Reduce 500 ~ 600 g of the specimen to a fine powder and mix 5.0 g 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 before extracting it for 30 minutes using ultrasonic waves. After transferring the hexane layer to a separating funnel, add 50 mL of hexane to the aqueous layer. Extract it by shaking, and repeat this process again. Collect the hexane layer from the second process and add it to the separating funnel. Add 50 mL of water to the hexane layer. 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 to 2 mL. Use a florisil cartridge that has been previously activated by eluting 10 mL of dichloromethane and 20 mL of hexane in order at the speed of 2 ~ 3 drops per second.
Pour the extracted solution into the previously activated cartridge and elute 20 mL of hexane dichloromethane mixture (3: 1) at the speed of 2 ~ 3 drops per second. After drying the elute with nitrogen gas in water-bath at below 35 °C and dissolving the residues in 1mL of acetonitrile, filter it with a membrane filter (pore size of 0.45 μm or less) to make a test solution. Take an appropriate amount of standard benzopyrene and standard 3-methylcholanthrene, dissolve them in acetonitrile separately to make 1 μg/mL undiluted standard solution and internal undiluted standard solution. Store the undiluted standard solution and internal undiluted standard solution at 5 ~ 15 °C and use them within 30 days.
Take an appropriate amount of the undiluted standard solution and internal undiluted standard solution, and dilute them with acetonitrile to make solutions that contain 3, 5, 10, 20 and 40ng of benzopyrene as well as 50 ng of an internal standard matter respectively. Use these as standard solutions. If the concentration of the test solution is outside the range of the calibration curve, adjust the concentration to within the range of the calibration curve. With 10 μL of the test solution and 10 μL of the standard solution carry out liquid chromatography under the following conditions. Plot a calibration curve by putting the ratio $[A_S/A_{IS}]$ of the peak area of benzopyrene to the peak areas of the internal standard matter, obtained from each standard solution, on the Y-axis, and putting the concentration of benzopyrene on the X-axis. Find the concentration of benzopyrene by plugging the ratio $[A_{SAM}/A_{SAMIS}]$ of the peak area of benzopyrene to the peak areas of the internal standard matter, obtained from the test solution, on the Y-axis

A_S: peak area of the standard matter, calibration curve of standard solution
A_{IS}: peak area of the internal standard matter, calibration curve of standard solution
A_{SAM}: peak area of benzopyrene in test solution
A_{SAMIS}: peak area of internal standard matter in test solution

Internal standard solution 3-methylcholanthrene
Dissolve the standard solution in 1mL of acetonitrile to make a 1mL/50ng solution.

Reagents and test solutions Water used for this test must be distilled 3 times or more, and test solution must be for testing agrochemical residues or of a higher grade.

Conditions
Detectors: fluoro spectrophotometer (excitation wavelengths 294 nm, fluorescence wavelengths 404 nm)
Column: Supelcosil LC-PAH (4.6 × 250 mm, 5 μm) or equivalent
Column temperature: 37 °C
Mobile phase: acetonitrile-water mixture (8: 2)
Flow rate: 1.0 mL/min.

Loss on Drying
Unless specified, carefully weigh 2 ~ 6g of the test specimen using a weighing bottle. After drying the specimen at 105 °C for 5 hours and cooling it in a desiccator (silica gel), weigh it again. Dry this at 105 °C again for 1 hour and weigh it. Do this every hour until no changes occur in weight. Reduction in weight is the loss on drying. If there is a limit in drying time, loss on drying (%) can be calculated by the reduction in weight during the set period of time.

Total ash Preheat a platinum, quartz or ceramic crucible at 500 ~ 550 °C for 1 hour and leave it to cool down. Carefully weigh the crucible. Unless specified, place 2 ~ 4g of the test specimen in the crucible and weigh it. If necessary, cover the crucible fully or partially with a lid. Start to heat the crucible at a low heat and gradually increase heat until the temperature reaches 500 ~ 550 °C. Incinerate the specimen by heating it for 4 hours or longer at 500 ~ 550 °C until there is no carbide left. Leave it to cool down and weigh the ash. Incinerate it again and repeat this process until no changes occur in weight. Carefully weigh the ash to find ash content (%). If a constant weight cannot be achieved using this method, put in boiling water and lixiviate. Filter it using a quantitative filter paper, and hard-burn the residue until cardible disappears, including the insoluble matter on the filter paper. Add filtered water to this and dry it through evaporation before hard-burning it. Let it cool down and then weigh it to find ash content (%). If carbide still 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 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. After drying it with the filter paper and hard-burning it for 3 hours using the same method used for ashes. Let it cool down in a desiccator (silica gel) before weighing it to find the acid-insoluble ash content (%). If the calculated value is larger than the standard value, hard-burn it again until there is no change in weight.

Extract content Carry out the following methods.

1) Diluted ethanol-soluble extraction

Unless specified, put 2.3 g of the test specimen into an appropriate flask and add 70 mL of diluted ethanol. Lixiviate it for 5 hours, occasionally shaking it to mix. Let it stand for 16 ~ 20 hours before filtering it. Rinse off the residue from the flask with diluted ethanol until 100 mL of filtered solution is obtained. After evaporate-drying 50mL of the filtered solution in water-bath and drying it at 105 °C for 4 hours, cool it down in a desiccator (silica gel). Measure its weight and calculate the amount of the diluted ethanol extraction by multiplying the weight by 2. Calculate the extraction amount (%) against the amount of the dry specimen, obtained from the loss on drying.

2) Water-soluble extraction

Follow the same method used 1) Diluted ethanol. But use water instead of diluted ethanol to measure the weight. Multiply the weight by 2 to find the amount of water extraction. Calculate the extraction amount (%) against the amount of the dry specimen, obtained from the loss on drying.

3) Ether-soluble extraction

Unless specified, dry the test specimen in a desiccator (silica gel) for 48 hours and put 2 g in to an appropriate flask. Boil it at a low heat in water-bath for 4 hours before cooling it down using a reflux condenser and filtering it. Rinse the residue in the flask with ether until 100mL of filtered solution is obtained. Evaporate and dry 50mL of the filtered solution in a water-bath and dry it in a desiccator (silica gel) for 24 hours. Carefully measure the weight 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 clauses of the relevant regulations for each drug and medication, into a hard-glass flask with a capacity of 1000mL. Add water 5 ~ 10 time of the specimen and install an essential oil meter (Fig.1). Connect a reflux condenser to the left end of the meter (Fig.2) and carefully boil it in oil-bath at 130 ~ 150 °C. Fill the scaled tube of the meter with water to the datum line and add 2.0 mL of xylene. Unless specified, boil it for 5 hours and let it stand briefly. After opening the cork of the meter and draining the water slowly, until the top end of the oil layer reaches the preliminary line on the tube. Let it stand for 1 hour at room temperature before lowering the oil layer to the zero line on the tube. Measure the amount of the oil (mL) at room temperature and take away the amount of xylene to find the amount of essential oil content in the herbal medicine.

52. Test for Histamine

Test for Histamine is a method to detect histamine and histamine-like substances in drug substance. Use the test conditions specified in the individual monograph. Unless otherwise specified, proceed as follows.

Standard solution of histamine Weigh accurately an appropriate amount of histamine phosphate RS, dissolve in Water for injection or Isotonic Sodium Chloride Injection to make a solution so that eachmL contains 1 μg of histamine ($C_5H_9N_3$), and use this solution as the histamine standard solution.

Test animals Use adult healthy cat.

Test solution and amount of test solution Test solution is specified in the individual monograph. Unless otherwise specified, use Water for Injection or Isotonic Sodium Chloride Injection to dissolve in the preparation of test solution and to suspend provided no hinder occurs in a test. If a solvent is specified to use for the preparation of test solution, a suspension in the solvent is acceptable for the test provided no hinder occurs. Unless otherwise specified, the amount of test solution is 1.0mL per kg of body weight. The amount of test solution specified in the individual monograph is the amount of administration per kg of test animal.

Procedure Weigh the body weight of test animal, inject the solution of Phenobarbital, sodium hexobarbital or sodium pentobarbital into abdomen for general anesthesia. Expose dextroversion artery, separate all tissue around vagus nerve by using a mess, and insert a cannula. Then expose vena femoralis, operate a recording chymograph to record a change in blood pressure, and confirm to reach a constant blood pressure. Inject histamine standard solution into vena femoralis as follows, and determine the sensitivity of test animal. Inject 0.05 mL, 0.1 mL, and 0.15 mL each of histamine standard solution with not less than 5 minutes interval in a serial. After not less than 5 minutes interval, repeat the serial injection. Discard the reading of the first serial, observe the drop of blood pressure to be almost constant with an amount of histamine and stop the injection, and take this amount as the standard amount of test solution which evoke not less than 2.67 kPa drop of blood pressure due to 0.1 ug histamine per Kg. Inject an appropriate amount of test solution for the body weight of test animal into vena femoralis, and observe the change of blood pressure for 5 minutes. A significant drop of blood pressure occurs, repeat the test on the test animal

with the histamine standard solution, then inject the test solution in the same manner and identify. If the test animal is stable enough, the test animal can be used for not less than two samples.

Decision of test results Decide that it meets the requirements, when the drop of blood pressure caused by test solution is not greater than that of blood pressure caused by 0.1 ug histamine per kg.

53. Test for Metal Particles

Test for Metal Particles is to examine the existence of foreign metal particles in the Ophthalmic Ointments.

Preparation of test sample

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude 5 g each of their contents into separate flat-bottomed Petri dishes 60 mm in diameter. Cover the dishes, and heat between 85 °C and 110 °C for 2 hours to dissolve bases. Allow the samples to cool to room temperature without agitation to be solidified. When the amount of the content is less than 5 g, extrude the contents as completely as practicable, and proceed in the same manner as described above.

Procedure

Invert each dish on the stage of a suitable microscope previously adjusted to provide not less than 40 time magnifications and equipped with an eyepiece micrometer. Each dish is illuminated from above 45° relative to the plane of the dish. Examine the entire bottom of each dish for metal particles and record the total number of particles not less than 50 μm diameter. Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with bottom.

Evaluation

To be suitable, the total number of metal particles counted is not more than 50 and the number of Petri dishes which has more than 8 particles is not more than 1. When it is not suitable, another 20 samples are prepared and tested as mentioned above, and the total number of metal particles, of which diameter is not less than 50 m, is not more than 150 from all of 30 samples, and the number of Petri dishes which has more than 8 particles is not more than 3, to be suitable.

54. Test for Rubber Closure for Aqueous Infusions

The rubber closure for aqueous infusions means a rubber closure (including material coated or laminated with a stuff like plastics) used for a container for aqueous infusion having a capacity of 100mL or more, and is in direct contact with the contained aqueous infusion. The rubber closure when in use does not interact physically or chemically with the contained medicament to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

(1) Cadmium

Wash the rubber closures with water, dry at room temperature, cut into tiny pieces, mix well, place 2.0 g of them in a crucible of platinum or quartz, moisten them with 2 mL of sulfuric acid, heat gradually to dryness, and ignite between 450 °C and 500 °C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4mL of hydrochloric acid, heat on a water-bath to dryness, add 1 to 5mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1mL of a mixture of citric acid solution (1 in 2) and hydrochloric acid (1:1), and 0.5 to 1mL of warmed ammonium acetate solution (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained, add 10mL of a solution of ammonium citrate (1 in 4), 2 drops of bromothymol bule TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10mL of ammonium sulfate solution (2 in 5) and water to make 100mL.

Next, add 20 mL of sodium diethyldithiocarbamate solution (1 in 20), mix, allow to stand for a few minutes, add 20.0mL of methyl isobutyl ketone, and mix by vigorous shaking. Allow to stand to separate the methyl isobutyl ketone layer from the solution, filter if necessary, and use as the test solution. On the other hand, to 10.0mL of standard cadmium solution add 10 mL of a solution of diammonium hydrogen citrate (1 I n4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the test solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry under the following conditions, using the sample solution and the standard solution, respectively. The absorbance of the test solution is not more than that of the standard solution.

Gas: Dissolved Acetylene or hydrogen- Air.
Lamp: Cadmium hollow-cathode lamp.
Wavelength: 228.8 nm.

(2) Lead

To 1.0 mL of the standard lead solution, add 10mL of a solution of diammonium hydrogen citrate(1in4) and 2 drops of bromothymol blue TS, proceed as directed for the test solution under 1), and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry under the following conditions, using the test solution obtained in 1) and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Dissolved Acetylene or hydrogen- Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.3 nm.

(3) Extractable substances

Wash the rubber closures with water, and dry at room temperature. Place them in a glass container, add water exactly 10 times the mass of the test material, close with a suitable stopper, heat at 121 °C for 1 hour in an autoclave, take out the glass container, allow to cool to room temperature, then take out immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution, respectively.

(i*) Description* The test solution is clear and colorless. Read the transparency of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0 %.

(ii) *Foamtest* Place 5mL of the test solution in a glass-stoppered test tube of about 15 mm in inner diameter and about 200 mm in length, and shake vigorously for 3 minutes. The foam arisen disappears almost completely within 3 minutes.

(iii) *pH* To 20 mL each of the test solution and the blank solution add 1.0 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

(iv) *Zinc* To 10.0mL of the test solution add diluted dilute nitric acid (1 in 3) to make 20 mL, and use this solution as the test solution. Further, to 1.0 mL of standard zinc solution for Atomic Absorption Spectrophotometry, add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry, using these solutions, under the following conditions. The absorbance of the test solution is not more than that of the standard solution.

Gas: Dissolved Acetylene - Air.
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

Standard Zinc Solution for Atomic Absorption Spectrophotometry: Measure exactly 10 mL of the standard zinc stock solution, and add water to make exactly 1000 mL. Prepare before use. 1 mL of this solution contains 0.01 mg of zinc (Zn).

(v) *Potassium Permanganate-reduciblesubstances* Measure 100 mL of the test solution in a glass-stoppered, Erlenmyer flask, add 10.0 mL of 0.002 mol/L potassium permanganate VS and 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference inmL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

(vi) *Residue on evaporation* Measure 100 mL of the test solution, evaporate on a water-bath to dryness, and dry the residue at 105 °C for 1 hour. The mass of the residue is not more than 2.0 mg.

(vii) *UV spectrum* Read the absorbance o the test solution between 220 nm and 350 nm against the blank solution as directed under the Ultraviolet-visible Spectrophotometry. The absorbance is not more than 0.20.

(4) Acute systemic toxicity

Wash the rubber closures with water and Waters for Injection successively, and dry under clean conditions at room temperature. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution 10 times the mass of the test material, place stopper adequately, heat in an autoclave at 121 °C for 1 hour, take out the glass container, and allow it to be cooled to room temperature. The resulting solution is used as the test solution. The blank solution is prepared in the same manner.

(i) *Test procedures Test animals*: Use healthy male mice of inbred strain or from a closed colony, weighing 17 to 23 g.

Procedure: Separate the animals into two groups of 10 mice, and inject intravenously 50mL each of the solutions per kg body weight.

(ii) *Interpretation* Observe the animals for 5 days after injection: During the observation period, none of the animals treated with the test solution show any abnormality or death.

(5) Pyrogen test

The test solution specified in 4) meets the requirements of the Pyrogen Test as does the blank solution.

(6) Hemolysis test

When 0.1mL of defibrinated blood or rabbit is added to 10mL of the test solution specified in 4) and the mixture is allowed to stand at 37 °C for 24 hours, hemolysis is not observed. Perform the blank test in the same manner, using 10mL of the blank solution.

55. Test for Total Organic Carbon

Test for Total Organic Carbon is a method for measuring the amount of organic carbon, which forms organic compounds, in water. Normally, organic carbon can be oxidized to carbon dioxide by a dry decomposition method, where organic compounds are oxidized by combustion, or by a wet decomposition method, where organic compounds are oxidized by applying ultraviolet rays or by adding oxidizing agent. The amount of carbon dioxide generated in the decomposition process is measured using an appropriate method such as infrared gas analysis, electric conductivity measurement, or resistivity measurement. The amount of organic carbon in water can be calculated from the amount of carbon dioxide measured in one of the above methods.

There are two types of carbon in water: organic carbon and inorganic carbon. For measuring the amount of organic carbon, two approaches can be taken. One method is to measure the amount of total carbon in water, then to subtract the amount of inorganic carbon from that of total carbon. The other method is to remove the amount of remaining organic carbon.

Instrument

The instrument consists of a sample injection port, a decomposition device, a carbon dioxide separation block, a detector, and a data processor or a recorder. The instrument should be capable of measuring the amount of organic carbon down to 0.050 mg/L.

The sample injection port is designed to be able to accept a specific amount of sample injected by a microsyringe or other appropriate sampling devices. The decomposition device for the dry decomposition method consists of a combustion tube and an electric furnace to heat the sample. Both devices are adjusted to operate at specified temperatures. The decomposition device for the wet decomposition method consists of an oxidizing reaction box, an ultraviolet ray lamp, a decomposition aid, injector, and a heater. The decomposition device for either method should be capable of generating not less that 0.450 mg/L of organic carbon when using a solution of sodium dodecylbenzenesulfonate (theoretical value of total organic carbon in this solution is 0.806 mg/L) as the sample. The carbon dioxide separation block removes water from carbon dioxide formed in the decomposed gas. An infrared gas analyzer, electric conductivity meter or specific resistance meter is used as the detector which converts the concentration of carbon dioxide into electric signal. The data processor calculates the concentration of the total organic carbon in the sample based on the electric signal converted by the detector. The recorder records the electric signal intensity converted by the detector.

Reagents and standard solutions

(1) ***Water used for measuring organic carbon*** (water for measurement): This water is used for preparing standard solutions or decomposition aid or for rinsing the instrument. The amount of organic carbon in this water, when collected into a sample container, should be not more than 0.250 mg/L.

(2) ***Standard potassium hydrogen phthalate solution*****:** The concentration of this standard solution is determined as specified for the instrument. Dry potassium hydrogen phthalate (standard reagent) at 105 ºC for 4 hours, and allow it to cool in a desiccator (silica gel). Weigh accurately a prescribed amount of dried potassium hydrogen phthalate, and dissolve it in the water for measurement to prepare the standard solution.

(3) ***Standard solution for measuring inorganic carbon*****:** The concentration of this standard solution is determined as specified for the instrument. Dry sodium hydrogen carbonate in a desiccator (sulfuric acid) for not less than 18 hours. Dry sodium carbonate decahydrate separately between 500 ºC and 600 ºC for 30 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately prescribed amounts of these compounds so that the ratio of their carbon content is 1 : 1, and dissolve them in the water for measurement to prepare the standard solution.

(4) ***Decomposition aid***: Dissolve a prescribed amount of potassium peroxodisulfate or other substances that can be used for the same purpose, in the water for measurement up to the concentration as specified for the instrument.

(5) ***Gas for removing inorganic carbon or carrier gas*****:** Nitrogen, oxygen, or other gases that can be used for the same purpose.

(6) ***Acid for removing inorganic carbon:*** Dilute hydrochloric acid, phosphoric acid or other acids that can be used for the same purpose with the water for measurement down to the concentration as specified for the instrument.

Apparatus

(1) ***Sample container and reagent container*****:** Use a container made of the material which does not release organic carbon from its surface, such as hard glass. Soak the container before use in a mixture of diluted hydrogen peroxide solution (1 in 3) and dilute nitric acid (1 : 1), and wash well with the water for measurement.

(2) ***Microsyringe***: Wash a microsyringe with a mixture of a solution of sodium hydroxide (1 in 20) and ethanol(99.5) (1 : 1),or diluted hydrochloric acid(1 in 4), and rinse well with the water for measurement.

Procedure

Employ an analytical method suitable for the instrument used. Calibrate the instruments using the standard potassium hydrogen phthalate solution with the test procedure specified for the instrument.

It is recommended that this instrument be incorporated into the manufacturing line of the water to be tested.

Otherwise, this test should be performed in a clean circumstance where the use of organic solvents or other substances that may affect the result of this test is prohibited, using a large sample container to collect a large volume of the water to be tested. The measurement should be done immediately after the sample collection.

(1) ***Measurement of organic carbon by subtracting inorganic carbon from total carbon***

According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of total carbon into the instrument from sample injection port, and decompose organic and inorganic carbon in the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of total carbon in the sample using a data processor or a recorder. Change the setting of the instrument for measuring inorganic carbon exclusively, and measure the amount of inorganic carbon

in the same manner as total carbon. The amount of organic carbon can be obtained by subtracting the amount of inorganic carbon from that of total carbon.

(2) *Measurement of organic carbon after removing inorganic carbon*

Remove inorganic carbon by adding the acid for removing inorganic carbon to the sample, followed by bubbling the gas for removing inorganic carbon (e.g. nitrogen) into the sample. According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, and decompose the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon in the sample using a data processor or a recorder.

For the instrument where the removal of inorganic carbon is performed in the instrument, first inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, according to the test procedure specified for the instrument used. Then, remove inorganic carbon by adding the acid for removing inorganic carbon to the sample in the decomposition device, followed by bubbling the gas for removing inorganic carbon into the sample. Decompose organic carbon, detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon using a data processor or a recorder.

56. Test Methods for Plastic Containers

Test Methods for Plastic Containers may be used for designing and quality assurance of plastic containers. Not all tests described here will be necessary in any phases for any containers. On the other hand, the set does not include sufficient number and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered, if necessary.

Combustion Tests

(1) *Residue on ignition* Weigh accurately about 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 Limit Test. Prepare the control solution with 2.0 mL of standard lead solution.

(3) *Lead*

Method 1: Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, moisten with 2 mL of sulfuric acid, heat slowly to dryness, then heat to combustion at between 450 °C and 500 °C. Repeat this procedure, if necessary. After cooling, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, evaporate to dryness on a waterbath, then add 1 to 5 mL of hydrochloric acid, and warm to dissolve. Then add 0.5 to 1 mL of a mixture of a solution of citric acid (1 in 2) and hydrochloric acid (1 : 1), and add 0.5 to 1mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter, if insoluble matter remains. To the obtained filtrate, add 10mL 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 solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium diethyldithiocarbamate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter, if necessary, and use the layer as the test solution. Separately, to 2.0 mL of standard lead solution, add water to make exactly 10 mL. To 1.0mL of this solution, add 10mL of diammonium hydrogen citrate solution (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the test solution, and use the solution so obtained as the standard solution. Perform the test with the test solution and the standard solution according to Atomic Absorption Spectrophotometry under the following conditions, and determine the concentration of lead in the test solution

Gas: Dissolved Acetylene or hydrogen—Air.
Lamp: Lead hollow cathode lamp.
Wavelength: 283.3 nm.

Method 2: Cut a container into pieces smaller than 5-mm square, take 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanone and 0.1mL of nitric acid, and warm to dissolve. To this solution, add 96 mL of methanol gradually to precipitate a resinous substance, and filter 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 about 10 mL under reduced pressure. Transfer into a separator, add 10 mL of ethyl acetate and 10 mL of water, shake vigorously, and allow to stand to separate the water layer. Evaporate the water layer to dryness, add 5mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1mL of a mixture of a solution of citric acid (1 in 2) and hydrochloric acid (1:1), and add 1mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3), if insoluble matter remains. To the solution so obtained, add 10mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and then add ammonia TS until the color of the solution changes from yellow to green. Further add 10mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium diethyldithiocarbamate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake

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, pipet exactly 5mL of standard lead solution, add water to make exactly 50mL, and to 2.0 mL of this solution, add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the test solution, and use the solution so obtained as the standard solution. Perform the test with the test solution and the standard solution, respectively, according to Atomic Absorption Spectrophotometry under the conditions described in Method 1, and determine the oncentration of lead in the test solution.

(4) *Cadmium Method 1*: To 2.0 mL of standard cadmium solution, add 10mL of a solution of ammonium citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the test solution in Method 1, under (3), and use the solution so obtained as the standard solution. Perform the test with the test solution obtained in Method 1, under (3) and the standard solution according to Atomic Absorption Spectrophotometry under the following conditions, and determine the concentration of cadmium in the test solution.

Gas: Dissolved Acetylene or hydrogen—Air.
Lamp: Cadmium hollow cathode lamp.
Wavelength: 228.8 nm.

Method 2: To 2.0 mL of standard cadmium solution, add 10mL of a solution of ammonium citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the test solution in Method 2 under (3), and use the solution so obtained as the standard solution. Perform the test with the test solution obtained in Method 2, under (3) and the standard solution 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 by gentle heating in a muffle furnace, occasionally adding drop-wise a mixture of sulfuric acid and nitric acid (1 : 1) until the content changes to a clear, light brown solution. Then heat until the color of the solution changes to a clear, light yellow, and heat to slowly concentrate to practical dryness. After cooling, dissolve the residue in 5 mL of hydrochloric acid by warming, and after cooling, add water to make exactly 10 mL. Pipet 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 10mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia water (28) (1 in 2), and measure the volume consumed for neutralization as amL. To the volumetric flask, A, add potassium permanganate TS drop-wise until a slight pale red color develops, and add a small amount of L-ascorbic acid to decolorize. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of a solution of citric acid (1 in 10), amL of diluted ammonia water (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake well, then allow to stand for about 20 minutes, and use this solution as the test solution. Separately, pipet exactly 1.0mL of standard tin solution, add 5mL of water, add potassium permanganate TS drop-wise until a slight pale red color develops, proceed in the same manner as for the test solution, and use this solution as the standard solution. Determine the absorbances of the test solution and the standard solution, respectively, according to Spectrophotometry at 510 nm, using water as the blank.

Extractable substances

Cut the container at homogeneous regions of low curvature and preferably the same thickness, gather pieces to make a total surface area of about 1200 cm2 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 at room temperature. Place these strips in a 300 mL hard glass vessel, add exactly 200 mL of water, and 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 falls to room temperature, and use the content 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 volume of water used 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 among the following conditions: at 100 ± 2 °C for 2 ± 0.2 hours, at 70 ± 2 °C for 24 ± 2 hours, at 50 ± 2 °C for 72 ± 2 hours, or at 37 ± 1 °C for 72 ± 2 hours. Prepare the blank solution with water in the same manner. For containers made of composite plastics, water is used as the blank solution. Perform the following tests with the test solution and the blank solution:

(1) ***Foaming test*** Place 5mL of the test solution in a glass-stoppered test tube, about 15 mm in inside diameter and about 200 mm in length, shake vigorously for 3 minutes, and measure the time needed for almost complete disappearance of the foam thus generated.

(2) ***pH*** To 20 mL each of the test solution and the blank solution, add 1.0 mL of a solution of potassium chloride (1 in 1000), and obtain the difference in the reading of pH between these solutions.

(3) ***Potassium permanganate reducing substances*** Place 20 mL of the test solution in a glass-stoppered, conical flask, add 20.0 mL of 2 mmol/L potassium permanganate VS and 1mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potas-

sium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the test in the same manner, using 20.0 mL of the blank solution, and obtain the difference of the consumption of 2 mmol/L potassium permanganate VS between these solutions.

(4) *Ultraviolet-visible spectrum* Record the maximum absorbances between 220 nm and 240 nm, and between 241 nm and 350 nm of the test solution against the blank solution as directed under the Untraviolet-visible Spectrophotometry.

(5) *Residue on evaporation* Evaporate 20 mL of the test solution on a waterbath to dryness, and weigh the residue after drying at 105 °C for 1 hour.

Test for fine particles

Rinse thoroughly with water the inside and outside of containers to be used for the tests, fill the container with the labeled volume water for particle matter test or 0.9 w/v % sodium chloride solution, adjust so that the amount of air in the container is about 50mL per 500mL of the labeled volume, stopper tightly, and heat at 121 °C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121 °C, employ a suitable temperature-time combination as directed under Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle for filterless infusion into the container through the rubber closure of the container, take the effluent while mixing gently in a clean container for measurement, and use this solution as the test solution.

Fine particle test—Counting of the fine particles must be performed in dustless, clean facilities or apparatus, using a light-shielded automatic fine particle counter. The sensor of the counter to be used must be able to count fine particles of 1.5 μm or more in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. For calibration of the diameter and number of particles, the standard particles for calibration of the light-shielded automatic fine particle counter should be used in suspension in water for particle matter test or 0.9 w/v % sodium chloride solution. Count five times the numbers of particles with diameters of 5 to 10 μm, 10 to 25 μm, and more than 25 μm while stirring the test solution, and calculate the average particle numbers of four counts, excluding the first, as the number of particles in 1.0 mL of the test solution.

Reagent — Water for particle matter test and 0.9 w/v % sodium chloride solution to be used for the tests should not contain more than 0.5 particles of 5 to10 μm in size per 1.0 mL.

Transparency test

(1) *Method 1* This can 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 of about 0.9 X 4 cm in size, immerse each piece in water filled in a cell for determination of the ultraviolet spectrum, and determine the transmittance at 450 nm using a cell filled with water as a blank.

(2) *Method 2* (Sensory test) This can be applied to containers which have a rough or embossed surface. It can also be applied to testing of the transparency of containers in case where the turbidity of their pharmaceutical contents must be checked.

Reagents (i) *Formadin standard suspension* Dilute 15 mL of the formadin stock suspension with water to make 1000 mL use within 24 hours of preparation, Shake throughly befor use.

(ii) *Reference suspension* Dilute 50 mL of the formadin standard suspension with water to make 100 mL.

Tests (i) *Method 2A (with control)* Take 2 containers to be tested, and place in one of them the labeled volume of the reference suspension and in the other, the same volume of water. Show the two containers to five subjects, separately, who do not know which one is which, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) *Method 2B (without control)* Take 6 numbered containers to be tested, and place in three of them the labeled volume of the reference suspension and in the others, the same volume of water. Show each one of the containers at random order to five subjects, separately, who do not know which one is which, ask if it is turbid or not, and calculate the percentage of the answer that it is turbid (100X/15, X: number of containers judged as “being turbid” in each group.

Water vapor permeability test

(1) *Method 1* This test method is applicable to containers for aqueous injection. Fill the container with the labeled volume of water. After closing it hermetically, 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 record the value. Calculate the mass loss during storage.

(2) *Method 2* This test method is provided for evaluating moisture permeability of containers for hygroscopic drugs. Unless otherwise specified, perform the test according to 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 at 110 °C for 1 hour, and cool in a desiccator.

Procedure—Select 12 containers, clean their surfaces with a dry cloth, and close and open each container 30 times in the same manner. Ten among the 12 containers are used as “test containers” and the remaining two, as "control containers” A torque for closing screw-capped containers is specified in the table. Add desiccant to 10 of the containers, designated test con-

tainers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63 mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and desiccant; the layer of desiccant in such a container shall be not less than 5 cm in depth. Close each container immediately after adding desiccant, applying the torque designated in the table. To each of the control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the test containers, and close, applying the torque designated in the table. Record the mass of the individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest l mg if the container volume is 20 mL or more but less than 200mL, or to the nearest 10 mg, if the container volume is 200mL 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. Completely fill 5 empty containers with water or a non-compressible, free-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, inmL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

$$\text{mg/day/L} = \frac{1000}{14V} \times [(T_f - T_i)] - (C_f - C_i)]$$

V: Average volume (mL),

T_f-T_i: Difference between the final and initial masses of each test container (mg),

C_f-C_i: Average of the differences between the final and initial masses of the two controls (mg).

Table. Torque applicable to screw-type container

Closure diameter (mm)	Torque (N·cm)	Closure diameter (mm)	Torque (N·cm)
8	59	48	216-343
10	60	53	235-402
13	88	58	265-451
15	59-98	63	284-490
18	78-118	66	294-510
20	88-137	70	314-569
22	98-157	83	363-735
24	118-206	86	451-735
28	137-235	89	451-794
30	147-265	100	510-794
33	167-284	110	519-794
38	196-294	120	618-1069
43	196-304	132	677-1069

Leakage test

Fill a container with a solution of fluorescein sodium (1 in 1000), stopper tightly, place filter papers on and under 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

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable.

(1) *Cell line* The recommended cell line is L929 (American Type Culture Collection-ATCC CCL1). This cell line is subcultivated in Eagle's minimum essential medium added with fetal calf serum. It is cultivated with 5 ± 1 % carbon dioxide in concentration and 36 °C to 38 °C in temperature until the cell layer convers not less than 80 % of the plate. Check whether the cell layer is homogeneous and regular when the cell-culture medium is examined under a microscope. Other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 cells.

(2) *Culture medium* Eagle's minimum essential medium prepared as follows shall be used. Dissolve the chemicals listed below in 1000mL of water. Sterilize the solution by autoclaving at 121 °C for 20 minutes. Cool the solution to room temperature, and add 22mL of sterilized sodium bicarbonate solution and 10mL of sterilized glutamine solution. To the resultant solution, add fetal calf serum (FCS) to make 10 vol % FCS in the medium.

Sodium chloride	6.80 g
Ppotassium chloride	400 mg
Sodium digydrogen 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
Citric acid	75.0 mg
Sodium citrate (hexahydrate)	100 mg
Choline bitaetrate	1.8 mg
Folic acid	1.0 mg
Myo-inositol	2.0 mg
Nicotinamide	1.0 mg

Calcium D-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

(i) *Reagents*

Sodium bicarbonate solution Dissolve 10 g of sodium bicarbonate in water to make 100mL. Sterilize the solution either by autoclaving in a well-sealed container at 121 °C for 20 minutes or by filtration through a membrane filter with a nominal pore diameter of 0.22 μm or less.

Glutamine solution Dissolve 2.92 g of L-glutamine in water to make 100mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

Phosphate buffer solution (PBS) 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 1000mL. Sterilize the solution by autoclaving at 121 °C for 20 minutes.

Trypsin solution Dissolve 0.5 g of trypsin, 0.2 g of tetrasodiumethylene diaminetetraacetate in phosphate buffer solution to make 1000mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

Formaldehyde solution Dilute formalin with water by a factor of ten.

Giemsa's stain solution Dilute a commercially available Giemsa's test solution with the diluent by a factor of fifty. Prepare before use.

Diluent Dissolve 4.54 g of potassium dihydrogen phosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000mL.

(ii) *Devices and instruments*

Pipets Pasteur pipet, pipet for partial delivery, measuring pipet for partial delivery, and dispenser with microtip.

Screw-capped glass bottles 50 to 1000mL volume.

Sterile disposable centrifuge tubes 15 and 50mL volume.

Sterile disposable tissue culture flasks with a flat growth area of approximately 25 or 75 cm^2.

Sterile disposable multiple well plates (24 wells).

Microscope Inverted microscope and stereomicroscope.

Carbon dioxide incubator Maintain the conditions as follows: Temperature, 37 °C; CO2 gas concentration, 5 %.

(iii) *Materials and substances*

Negative control material Polyethylene film.

Positive control material (A) Polyurethane film containing 0.1 percent zinc diethyldithiocarbamate.

Positive control material (B) Polyurethane film containing 0.25 percent zinc dibutyldithiocarbamate.

Control substances Zinc diethyldithiocarbamate (reagent grade) and zinc dibutyldithiocarbamate (reagent grade).

(iv) *Test procedure*

Sample preparation When the material of the container consists of a single homogeneous layer, subdivide the cut pieces of a container into pieces of the size of approximately 2 X 15 mm and subject the pieces to the test. When the material of the container has multiple layers, such as laminated and coated materials, prepare cut pieces with a surface area of one side of 2.5 cm^2 and subject the pieces to the test without subdividing them into smaller pieces.

Preparation of test solutions Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable 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. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube, add the culture medium in a proportion of 10mL to one gram or 2.5 cm^2 (one side) of the sample, loosely cap the bottle or tube, and allow to stand in a humidified incubator for 24 hours. Transfer the culture medium extract, which is designated 100 % test solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100 % test solution with culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50 %, 25 %, 12.5 %, 6.25 %, 3.13 %, and so on.

Preparation of cell suspension Remove the culture medium from the maintained cell culture flask and rinse the cells with an appropriate volume of PBS by gentle rotation of the flask two or three times, and discard the PBS. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the flask and place the flask in a humidified incubator for one or two minutes. After confirming detachment of the cell layer from the bottom of the flask by using a microscope, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the flask by using a Pasteur pipet. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge the tube at 800 to 1000 revolutions per minute for 2 to 5 minutes. Discard the supernatant, resuspend the cells in an appropriate volume of PBS by pipetting, using a Pasteur pipet, and centrifuge the tube again. Discard the PBS, and add an appropriate volume of fresh culture medium to the flask. Resuspend the cells by pipetting and make a single cell suspension. Determine the cell concentration using a hemocytometer.

Cytotoxicity testing Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 10^2 cells/mL. Place a 0.5mL aliquot of the diluted cell suspension on

each well of a sterile disposable multiple well plate. Incubate the plate in the humidified incubator for 4 to 6 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5mL aliquot of the test solution or fresh medium to quadruplicate wells. Place the plate immediately in the humidified incubator and incubate the plate for the appropriate period: 7 to 9 days for L929 cells. After the incubation, discard the medium from the plate, add an appropriate volume of dilute formaldehyde TS to each well and allow the plate to stand for 30 minutes to fix the cells. Discard the dilute formaldehyde solution from each well and add an appropriate volume of dilute Giemsa's stain solution to each well. After ensuring good staining of the colonies, discard the stain solution from the wells and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the test solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the colony formation rate (%) for each extract concentration of the test solution. Plot the extract concentration (%) of the test solution on a logarithmic scale and the colony formation rate on an ordinary scale on semi-logarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the percent extract concentration which inhibits colony formation to 50 %, IC_{50}(%), from the inhibition curve. It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable control materials or substances in the test system, if necessary.

Plastic Containers for Aqueous Injections

Plastic containers for the aqueous injections do not interact with pharmaceuticals contained therein to alter the efficacy, safety or stability, and do not permit the contamination with microorganisms. The containers meet the following requirements.

1) *Polyethylene or polypropylene containers for aqueous injections*

The containers are made of polyethylene or polypropylene and free from any adhesive.

(1) *Transparency* The containers have a transmittance of not less than 55 %, when tested as directed in Method 1 under the Transparency test. When Method 1 cannot be applied, test according to the Method 2B of the Transparency test is used. In this case, the rate that the water-containing container is judged as "being turbid" is less than 20 %. And the rate that the reference suspension-containing containers judged as "being turbid" is not less than 80 percent.

(2) *Appearance* The containers do not have strips, cracks, bubbles, or other faults which cause difficulties in practical use.

(3) *Water vapor permeability* Proceed as directed in Method 1 of the Water vapor permeability test. The loss of mass is not more than 0.20 %.

(4) *Heavy metals* The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(5) *Lead* Perform the test as directed in Method 1. The absorbance of the test solution is not more than that of the standard solution.

(6) *Cadmium* Perform the test as directed in Method 1. The absorbance of the test solution is not more than that of the standard solution.

(7) *Residue on ignition* The residue is not more than 0.10 %.

(8) *Extractable substances* (i) *Foaming test* The foam formed almost disappears within 3 minutes. (ii) *pH* The difference in the reading of pH between the test solution and the blank solution is not more than 1.5. (iii) *Potassium permanganate-reducing substances* The difference in the consumption of 0.002 mol/L potassium permanganate VS between the test solution and the blank solution is not more than 1.0 mL. (iv) *UV spectrum* The maximum absorbance between 220 nm and 240 nm is not more than 0.08, and that between 241 nm and 350 nm is not more than 0.05. (v) R*esidue on evaporation* Not more than 1.0 mg.

(9) *Cytotoxicity* IC_{50}(%)is not less than 90 %. The result obtained by the other standard methods is negative.

2) *Plastic containers for aqueous injections being not described above*

The containers meet the following specifications and other necessary specifications for their materials with regard to heavy metals, residue on ignition and extractable substances, etc.

(1) *Transparency* Proceed as directed in (1) under Polyethylene or polypropylene containers for aqueous injections.

(2) *Appearance* Proceed as directed in (2) under Polyethylene or polypropylene containers for aqueous injections.

(3) *Vaporpermeability* Proceed as directed in (3) under Polyethylene or polypropylene containers for aqueous injections.

(4) *Cytotoxicity* Proceed as directed in (9) under Polyethylene or polypropylene containers for aqueous injections.

57. Thermal Analysis

Thermal analysis is a term for a variety of analytical techniques to measure the physical properties of a substance as a function of temperature and/or time while varying the temperature of the substance by a certain temperature programming.

Among various physical properties, the techniques for detecting phase transitions such as those between solid and liquid phase (melting, freezing) and crystal polymorphism or thermal behaviors such as heat evolution or absorption accompanying thermal degradation or chemical reactions are called differential thermal analysis (DTA). The techniques for measuring heat quantity (enthalpy) changes are called differential scanning calorimetry (DSC) and the techniques for

measuring mass change of a sample caused by dehydration, adsorption, elimination or oxidation, etc., are called thermogravimetry (TG).

Among the above methods, thermogravimetry can be used as an alternative method for "Loss on Drying" or "Water Determination". However, it must be confirmed beforehand that no volatile component except water is present in the test specimen when TG is used as an alternative method for "Water Determination".

Method 1. Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC)

Apparatus

Apparatus for DTA or DSC is usually composed of a heating furnace, a temperature controller, a detector, an atmosphere control device, and a display/recorder.

(1) ***Differential Thermal Analysis (DTA)*** For this method, the heating furnace is used to heat or cool a sample and a reference material at a constant rate, and a detection device such as a thermocouple is used to record continuously the temperature difference evolved between the sample and the reference material as a function of time and/or temperature. a-Alumina for thermal analysis is used as a reference material.

(2) ***Differential Scanning Calorimetry (DSC)*** Two kinds of DSC apparatus, based on different principles are available as follow:

(i) *Input compensation-type differential scanning calorimetry (Input compensation DSC)* A sample and a reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference is detected by a device such as a platinum resistance thermometer and kept at null by controlling the heating unit with a compensation feedback circuit. The instrument is designed to measure and record continuously the balance of thermal energy applied to each furnace as a function of time and/or temperature.

(ii) *Heat flux-type differential scanning calorimetry (Heat flux DSC)* A sample and a reference material in twin furnace are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference is detected as a difference of heat flux and recorded. In heat flux DSC, thermal conductors are used so that the heat flux between the sample and the heat reservoir is proportional to the temperature between them. DSC signal between the reference and the heat reservoir is recorded by the same method. Usually, α-alumina for thermal analysis is used as a reference material, both in Input compensation DSC and in Heat flux DSC, but in some cases, an empty sample container can also be used without any reference material.

Procedure

A sample and a reference material are put in sample pans, and the furnace is heated or cooled by a controlled temperature program, while the temperature difference (DTA) or heat quantity change (DSC) developed between the sample and the reference is detected and recorded continuously. The apparatus is operated and the data are processed according to the instruction manual provided with the instrument.

A preliminary experiment within a wide temperature range (room temperature ~ the temperature at which degradation begins), in which scanning is done at a rapid heating rate (10 ~ 20 °C/minute) is needed to determine the appropriate temperature range of measurement, within which a predicted physical change such as melting or polymorphic phase transition will occur, and to confirm that unpredicted thermal changes are not induced in a specimen in that temperature range. Thereafter, tests should be performed at a heating rate, usually 2 °C/minute, in the chosen temperature range. However, when a clear heat change cannot be observed, such as in cases of glass-transition, the heating rate may be changed as appropriate for the kind of physical change under observation. By analyzing heat absorption or heat generation peaks from the obtained DTA curve or DSC curve, a quantity of heat change and/or a specific temperature (ignition, peak and end temperature) accompanying a physical changes, such as melting or polymorphic phase transition, is obtained.

Calibration of the apparatus

(1) ***Temperature calibration*** Temperature calibration for apparatus used for DTA or DSC can be performed by using melting points of pure metals or organic compounds or phase transition points of crystalline inorganic salts or oxides. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for the calibration.

(2) ***Heat quantity calibration*** For accurate estimation of quantity of heat change (enthalpy change) of a sample caused by temperature change, it is necessary to calibrate the apparatus by using appropriate reference materials. As indicated in the section of Temperature calibration, melting points of pure metals or organic compounds or phase transition points of crystalline inorganic salts or oxides are used for heat quantity calibration. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed.

Notes on operating conditions

When DTA or DSC measurements are made, the following operating conditions are recorded: sample size, choice of opened- or closed-type sample container, heating or cooling rate, measuring temperature range, and kind and flow rate of atmospheric gas.

Method 2 Thermogravimetry(TG)

Apparatus

The construction of a TG apparatus is fundamentally similar to that of DTA or DSC apparatus. However, the detector for TG is a thermobalance of hanging-, pan-, or horizontal-type. The TG apparatus is designed to detect mass changes of a sample placed at a fixed position on a thermobalance, caused by temperature change of the furnace under a certain temperature con-

trol program. Mass change with temperature and/or time is recorded continuously.

Procedure

A sample put in a sample container is placed at a fixed position of the thermobalance. The mass change of a sample is recorded continuously with temperature change by a certain temperature control program. The apparatus is operated and the data are processed according to the instruction manual provided with the instrument.

When TG is used as an alternative method for "Loss on Drying" or "Water Determination", the measurement starts at room temperature and ends at a temperature above which no further mass change due to drying and/or vaporization of water can be observed. The standard heating rate is usually 5 °C/minute. However, heating rate can be changed as necessary, depending on the kind of sample and the extent of the measuring temperature range. Further, dry air or dry nitrogen is usually pass through the heating furnace to ensure rapid elimination of evolved water or other volatile components and to avoid the occurrence of any chemical reaction such as oxidation. By analyzing mass-time and/or mass-temperature curve of the obtained TG curve, absolute mass change and/or relative mass change with respect to the initial quantity (%) caused by drying is calculated.

When the mass change caused by oxidation or degradation of a sample is measured, a specific temperature range has to be determined beforehand so that stable baselines can be obtained before and after a chemical reaction, and subsequent procedures are the same as described above.

Calibration of the apparatus

(1) ***Temperature calibration*** The Curie temperature of a substance such as Nickel for thermal analysis is used for temperature calibration for TG apparatus. In the case of a TG apparatus capable of simultaneously conducting DSC or DTA, the temperature calibration for TG is not needed when the temperature calibration under Method 1 is performed.

(2) ***Scale calibration and confirmation*** The scale calibration for TG is done by using reference masses for chemical balances and/or semimicrobalances in the targeted range of measurement, which is called a primary scale calibration. This primary scale calibration is done under ordinary temperature and pressure when the apparatus is set up initially and periodically, thereafter. When a sample is measured, scale calibration or confirmation is done by using Calcium Oxalate Monohydrate Reference Standard to take account of such effects as buoyancy and convection due to atmospheric gas flow under the real measurement conditions, which is called secondary scale calibration. This secondary scale calibration is done under standard operation conditions stated below or under other specified operation conditions by using Calcium Oxalate Monohydrate Reference Standard. When the difference of water content between the measured value and the certified one for the Reference Standard is less than 0.3 %, normal operation of the apparatus is confirmed. When the difference is more than 0.3 %, scale calibration is done, based on the certified water content of the Reference Standard.

Standard operation conditions:

Amount of Calcium Oxalate Monohydrate Reference Standard: 10 mg

Heating rate: 5 °C/minute

Temperature range: room temperature ~ 250 °C

Atmospheric gas: dried nitrogen or dried air

Flow rate of atmospheric gas, hanging- or pan-type balance: 40mL/minute

Horizontal-type balance: 100mL/minute

Notes on operating conditions

The following operating conditions are recorded: sample size, heating rate, measuring temperature range, and kind and flow rate of atmospheric gas, etc.

58. Thin-layer Chromatography

Thin-layer Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

Preparation of thin-layer plate

Generally, proceed by the following method.

A smooth glass plate of uniform thickness having a size of 5 cm × 20 cm or 20 cm × 20 cm is used for preparation of a thin-layer plate. Using a suitable apparatus, apply a water suspension of powdered solid substance for the stationary phase, as directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105 °C and 120 °C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate.

Preserve the dried plate with protection from moisture.

Procedure

Unless otherwise specified, proceed by the following method.

Designate a line at about 20 mm distant from the bottom of the thin-layer plate as the starting line, make round spots of 2 to 6 mm in diameter with the directed volumes of the test solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developing solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely,

and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.

$$R_f = \frac{\text{Distancd from the starting line to the center of the spot}}{\text{Distance from the starting line to the solvent front}}$$

When the solvent front has ascended from the starting line to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the R_f value by using the following equation:

59. Ultraviolet-visible Spectrophotometry

The Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light by substances, in a wavelength range between 200 nm and 800 nm for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used, proceed as directed under the Atomic Absorption Spectrophotometry.

When monochromatic light passes through a substance in solution, the ratio of the transmitted light power, P, to the incident light power, P_0, is called transmittance, t; transmittance expressed in percentage is called percent transmission T, and the common logarithm of the reciprocal of transmittance is called absorbance, A.

$$t = \frac{P}{P_0}$$

$$T = \frac{P}{P_0} \times 100 = 100 \times t$$

$$A = \log \frac{P_0}{P}$$

The absorbance A is proportional to the concentration c of a substance in the solution and the length l of the path length.

$$A = k \times c \times l \quad (k \text{ is a constant})$$

The absorbance, calculated on the basis that l is 1 cm and c is 1 mol/L, is called molar absorption coefficient ε. The molar absorption coefficient at the wavelength of maximum absorption is expressed as ε_{max}.

When a light beam passes through a solution of a substance, the absorbance of the sample differs depending on the wavelength of the light. So, ultraviolet-visible absorption spectrum is obtained by determining the absorbances of a light beam at various wavelengths and by graphically representing the relation between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelengths of maximum absorption, λ_{max}, and of minimum absorption, λ_{min}.

The absorption spectrum of a substance in solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance or to test the purity of a substance by comparing the spectrum of a sample within the specified wavelength range with the spectrum of reference standard, by determining the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specific wavelengths. Usually, the absorbance of a solution of a certain concentration is measured at the wavelength of the maximum absorption, for the purpose of assay.

Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of the absorbance. After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it is confirmed that the wavelength and the transmittance meet the specifications of the tests described below.

Using an optical filter for wavelength calibration, measure the transmittance in the vicinity of the standard wavelength value shown in the test result form, under the test conditions given in the test result form attached to each of the filters. When performing a test to determine the wavelength of minimal transmittance, the difference between the measured wavelength value and the standard wavelength value is within ±0.5 nm. When the measurement is repeated three times, each value obtained is within the mean ±0.2 nm. It is also possible to carry out the test using a low-pressure mercury lamp at bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm and 546.07 nm, or a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In case of these tests, the difference between the measured wavelength and the wavelength of the bright line is within ±0.3 nm. When the measurement is repeated three times, each value obtained is within the mean ±0.2 nm.

Using an optical filter for transmittance calibration, determine the transmittance at the standard wavelength value under the test condition given in the test result form attached to each of the filters. The difference between the measured transmittance value and the standard transmittance value is within the range of from 1 % larger of the upper limit to 1 % smaller of the lower limit for the relative precision shown in the test result form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmittance) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and should be within the mean ±0.004 when the absorbance is more than 0.500. In addition, it is desirable to confirm the linearity of transmittance at the same wave-

length using several optical filters for calibration with different transmittances.

Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed. Subsequently, allow the apparatus to stand for a certain time to confirm the stability. Then usually adjust the apparatus so that the transmittance is 0 % at measuring wavelength or over measuring wavelength range after closing shutter at the sample side of light path. Then open the shutter and adjust the transmittance to 100 % (the absorbance is zero). Adjusting the transmittance to 100 % is usually done by putting cells containing the control solution in both light paths. For the control solution, blank solvent is used, unless otherwise specified. Then perform the measurement with the cell containing the test solution, and read the absorbance at measuring wavelength, or acquire the spectrum over measuring wavelength range. Unless otherwise specified, a cell with pathlength of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in ultraviolet range and use a solvent which does not disturb accurate measurement.

Specific absorbance

The absorbance, calculated on the basis that l is 1 cm and c is the concentration, 1 w/v percent of the medicament, is called specific absorbance, and is expressed as $E_{1\,\mathrm{cm}}^{1\%}$

$$E_{1\,\mathrm{cm}}^{1\%} = \frac{A}{c \times l}$$

l : Length of the layer of solution (cm),
A : Absorbance value,
c : Concentration of the test solution (w/v percent).

The description in a monograph, for example, " $E_{1\,\mathrm{cm}}^{1\%}$ (241 nm): 500 to 530 (after drying, 2 mg, methanol, 200mL)", indicatesthat $E_{1\,\mathrm{cm}}^{1\%}$ is between 500 and 530 in the test, in which the sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200mL, then the absorbance of the solution is measured at a wavelength of 241 nm in a layer of 1 cm in length.

Identification

Prepare the test solution as directed in the monograph, and test as directed in the procedure. Usually, the test is performed by a single method or in a combination of the following methods using the absorbance or absorption spectrum obtained from test solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

(1) ***Identification using Reference Standard*** When the absorption spectrum obtained from the test solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be conformed.

(2) ***Identification using absorption wavelength*** When wavelengths of maximum absorption of spectrum obtained from the test solution match the wavelengths specified in the monograph, the identity of the sample may be confirmed.

(3) ***Identification using the ratios of the absorbances*** When the ratios of absorbances obtained at two or more wavelengths meet the specifications in the monograph, the identity of the sample may be confirmed.

Assay

Prepare the control solution, the test solution and the standard solution as directed in the monograph, measure the absorbances of the test solution and the standard solution according to the method described in the procedure and determine the amount of the substance to be assayed in the sample by comparing the absorbances. The assay can be done using the equation specified in the monograph and applying the measured absorbance values of the test solution and the standard solution.

60. Uniformity of Dosage Units

The term "Uniformity of dosage unit" is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, Unless otherwise specified elsewhere in this Pharmacopoeia.

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of a drug substance in each dosage unit.

Unless otherwise specified, Uniformity of Dosage Units Test is test method to expressed uniformity of contents of principal constituent in the preparations.

The Uniformity of Dosage Units specification is not intended to apply to suspension, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The Uniformity of Dosage Units can be demonstraited by either of two methods, Content uniformity or Mass variation (see Table 1).

The test for Content Uniformity of preparations presented in dosage units is based on the assay of the

individual contents of drug substance of a number of dosage units to determine whether the individual contents are within the limits set. The Content Uniformity method may be applied in all cases.
The test for Mass Variation is applicable for the following dosage forms:

(1) solutions enclosed in unit-dose containers and into soft capsules in which all components are perfectly dissolved;
(2) solids (including powders, granules and sterile solids) that are packaged in single-unit containers and contain no active or inactive added substances;
(3) solids (including sterile solids) that are packaged in singer-unit containers, with or without active or inactive added substances, that have been prepared from true solutions and freeze-dried in the final containers and are labeled to indicate this method of preparation; and
(4) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25 % or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, or in the case of film-coated tablets, the precoated tablets, except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting Content Uniformity requirements.
(5) Corresponding constituent of preparation that the content uniformity of major constituent is more than 10 % allowance variation to the expressed volume.
(6) Mass Variation Test is applied to the monograph.

The test for Content Uniformity is required for all dosage forms not meeting the above conditions for the Mass Variation test. Alternatively, products listed in item (4) above that do not meet the 25mg/25 % threshold limit may be tested for uniformity of dosage units by Mass Variation instead of the Content Uniformity test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2 %, based on process validation data and development data, and if there has been regulatory approval of such a change.
The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in Table 2.

Content uniformity

Select not less than 30 units, and proceed as follows for the dosage form designated. Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

Solid dosage forms-Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 2).

Liquid dosage forms-Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 2).

Calculation of Acceptance Value
calculate the acceptance value by the formula:

$$\left|M - \overline{X}\right| + ks$$

in which the terms are defined in Table 2.

Mass variation

Mass Variation is carried out based on the assumption that the concentration (mass of drug substance per mass of dosage unit) is uniform in a lot. Carry out an assay for the drug substances on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as % of label claim (see Calculation of the Acceptance Value). Select not less than 30 dosage units, and proceed as follows for the dosage form designated.
Uncoated or film-coated Tablets. Accurately weigh 10 tablets individually. Calculate the content, expressed as % of label claim, of each tablet from the mass of the individual tablets and the result of the assay. Calculate the acceptance value.
Hard Capsules. Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the respective gross mass. Calculate the drug substance content of each capsule from the mass of the individual capsules and the result of the assay. Calculate the acceptance value.
Soft Capsules. Accurately weigh the 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.
Solid dosage forms other than tablets and capsules Proceed as directed for Hard Capsules, treating each dosage unit as described therein. Calculate the acceptance value.
Liquid dosage forms Accurately weigh the amount of liquid that is removed from each of 10 individual containers in conditions of normal use. Calculate the drug substance content in each container from the mass of

product removed from the individual containers and the result of the assay. Calculate the acceptance value.

Calculation of Acceptance Value
Calculate the acceptance value as shown in Content Uniformity, except that the value of $\overline{X}$ is replaced with A, and that the individual contents of the dosage units are replaced with the individual estimated contents defined below.

$$x_i = w_i \times \frac{A}{\overline{W}}$$

x_1, x_2, …, x_n : individual estimated contents of the dosage units tested, where

w_1, w_2, …, w_n: individual masses of the dosage units tested,

A : content of drug substance (% of label claim) obtained using an appropriate analytical method.

W : mean of individual masses (w_1, w_2, …, w_n).

Criteria

Apply the following criteria, unless otherwise specified.

Solid and Liquid Dosage Forms The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to $L1$ %.If the acceptance value is greater than $L1$ %, test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage unit is less than or equal to $L1$ % and no individual content of the dosage unit is less than(1- $L2 \times 0.01$) M nor more than (1 + $L2 \times 0.01$)M is Calculation of Acceptance Value under Content Uniformity or under Mass Variation. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

In the case of equivalent to (5) of preparations by the application of mass variation.

1) *Granules, powers, syrups and liquid preparations in single-unit containers(divided)* Take 20 containers, weigh accurately mass of each container, calculate the mean mass and variation between the mean value and the mass of each container is not more than 10 % to meet the requirements. When the difference is more than 10 % in any of them, proceed to the following test with the content. Take 20 containers and weigh accurately mass of each container. In doing this, pay attention to relate the measured mass with each container by identifying each container with numbers or any other marks. Open the container, remove the content with a small brush or any suitable tool and weigh the mass of the empty container. Calculate the mass of the content by subtracting the mass of the empty container from the mass of each of the corresponding container. Calculate the mass of the content of each of 20 containers and the mean mass. In not more than 2 containers, variation between the mean value and mass of content of each container is more than 10 % and variation of more than 25 % is not observed, to meet the requirements.

2) *Tablets* Take 20 tablets, weigh accurately mass of each tablet, calculate the mean mass and variation between this mean value and the mass of each tablet is not more than the following values, the number of outliers is not more than 2 and the variation of more than 2 times of the following values is not observed, to meet the requirements.

Mean mass (g)	Variation (%)
Less than 0.12	10
Not less than 0.12 and less than 0.3	7.5
Not less than 0.3	5

3) *Suppositories* Take 20 suppositories, weigh accurately mass of each suppository, calculate the mean mass and variation between this mean value and the mass of each suppository is not more than 5 % and the number of suppositories of which variations are more than 5 % and not more than 7.5 % is not more than 2, to meet the requirements.

4) *Injections(to be reconstituted or suspended before use)* Take 10 injections, wash the exterior of the container of each injection after removal of paper label, if any, dry it completely in a desiccator to get a constant mass and weigh accurately the mass. Open carefully the containers, remove the contents, wash all parts of each container with water and ethanol, air dry, dry in a desiccator to get a constant mass and weigh accurately all parts of each of the opened containers. Calculate the net mass of the content as a difference of masses before and after removal of the contents. Calculate the mean mass of the contents of 10 injections and the variation between the mean value and each mass. The number of outliers by the following values is not more than 1 and the variation of more than 2 times of the following values is not observed, to meet the requirements.

Mean mass (g)	Variation (%)
Less than 0.015	15
Not less than 0.015 and less than 0.12	10
Not less than 0.12 and less than 0.3	7.5
Not less than 0.3	7

5) *Capsules* i) *Hard Capsules* Take 10 capsules and weigh accurately mass of each capsule, calculate the mean mass and variation between the mean value and the mass of each capsule is not more than 10 % to meet the requirements. When the difference is more than 10 % in any of them, take 20 capsules and weigh accurately mass of each capsule. In doing this, pay attention to relate the measured mass with each capsule by identifying each capsule with numbers or any other marks. Open the capsule, remove the content with a small brush or any suitable tool and weigh the mass of the empty capsule. Calculate the mass of the content by subtracting the mass of the empty capsule from the mass of each of the corresponding capsule. Calculate

the mass of the content of each of 20 capsules and the mean mass. In not more than 2 capsules, variation between the mean value and mass of content of each capsule is more than 10 % and variation of more than 25 % is not observed, to meet the requirements.

ii) *Soft Capsule* Take 20 capsules and weigh accurately mass of each capsule. In doing this, pay attention to relate the measured mass with each capsule by identifying each capsule with numbers or any other marks. Open the capsule, remove the content by washing with volatile solvent such as ether, remove the remaining solvent of the empty capsule by touching with a filter paper or any other suitable materials, leave the capsules in a room temperature to remove the remaining solvent. In doing this, prevent capsules from moisture absorption or drying. Weigh the mass of the empty capsule. Calculate the mass of the content by subtracting the mass of the empty capsule from the mass of each of the corresponding capsule. Calculate the mean mass of the contents of 20 capsules. In not more than 2 capsules, variation between the mean value and mass of content of each capsule is more than 10 % and variation of more than 25 % is not observed, to meet the requirements.

When the number of capsules of which variations are more than 10 % and not more than 25 % is 3 to 6, repeat the test with additional 40 capsules and calculate the mean value of the contents of total 60 capsules. In not more than 6 capsules, variation between the mean value and mass of content of each capsule is more than 10 % and variation of more than 25 % is not observed, to meet the requirements.

6) *Troches* Take 20 troches, weigh accurately masses and calculate the mean mass. The variation between the mean value and the mass of each troche is not more than 10 %, the number of outliers by 10 % is not more than 2 and variation of more than 20 % is not observed, to meet the requirements.

Table 1. Application of Content Uniformity(CU) and Mass Variation(MV) Test for Dosage Forms

Dosage forms	Type	Sub-type	Dose and ratio of drug substance	
			≥25 mg & 25 %	<25 mg & 25 %
Tablets	uncoated	-	MV	CU
	coated	film	MV	CU
		others	CU	CU
Capsules	hard	-	MV	CU
	soft	Suspension, emulsion, gel	CU	CU
		solutions	MV	MV
Solids in single unit containers (divided forms, lyophilized forms, et al.)	Single component		MV	MV
	Multiple component	Solution freeze-dried in final container	MV	MV
		others	CU	CU
Preparations in single unit containers (perfectly dissolved solutions)			MV	MV
Others			CU	CU

Table 2. Definition of Terms

Variable	Definition	Conditions	Value
$\overline{X}$	Mean of individual contents (x_1, x_2, …, x_n) expressed as a percentage of the label claim		
$x_1, x_2, \ldots, x_n$	Individual contents of the dosage units tested, expressed as a percentage of the label claim		
n	Sample size (number of dosage units in a sample)		
k	Acceptability constant	If n =10, then	2.4
		If 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 (the sample standard deviation expressed as a percentage of the mean)		$\frac{100s}{\overline{X}}$
M (case 1) To be applied when $T \le 101.5$	Reference value	If $98.5\% \le \overline{X} \le 101.5\%$, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X} \le 98.5\%$, then	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X} > 101.5\%$, then	$M = 101.5\%$ $(AV = \overline{X} - 101.5 + ks)$
M (case 2) To be applied when $T \le 101.5$	Reference value	If $98.5\% \le \overline{X} \le T$, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X} < 98.5\%$, then	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X} > T$, then	$M = T$ $(AV = \overline{X} - T + ks)$
Acceptance Value (AV)			General formula : $\left\|M - \overline{X}\right\| + ks$ [Calculations are specified above for the different cases.]
$L1$	Maximum allowed acceptance value		$L1$= 15.0 Unless otherwise specified.
$L2$	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than 0.75 M while on the high side, no dosage unit result can be greater than 1.25 M (This is based on an $L2$ alue of 25.0)	$L2$=25.0 Unless otherwise specified.
T	Target test sample amount at time of manufacture. Unless otherwise specified in the individual monograph, T is 100.0 %		

61. Viscosity Determination

Viscosity determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is

called a Newtonian liquid. The proportionality constant, η, is a characteristic of a liquid at a defined temperature and is called viscosity. The unit of viscosity is Pascal second(Pa·s), but usually milli-Pascal second(mPa·s) is used.

A liquid whose the slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. A sample of a non-Newtonian liquid shows different viscosity values according to the size of its slip velocity. The viscosity measured at a certain slip velocity is called an apparent viscosity, and information obtained on the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid. The quotient obtained by dividing the viscosity, η,by the density, η , of the liquid at the same temperature is called kinematic viscosity, v,of which the unit is meters squared per second(m^2/s), but usually millimeters squared per second(mm^2/s) is used. The viscosity of a liquid sample is determined by Method I or Method II as follows.

Method I. Viscosity measurement by capillary tube viscometer

For measuring the viscosity of a Newtonian liquid, a capillary tube viscometer is usually used, in which the down flowing time of a sample liquid, t(s), required for a definite volume of the sample to flow down through a capillary tube is measured and the kinematic viscosity, v, is calculated according to the following equation.

$$v = K \cdot t$$

Viscosity, η, is calculated according to the following equation. ρ (g/mL) is the density of sample liquid measured at the same temperature.

$$\eta = v \cdot \rho = K \cdot t \cdot \rho$$

The parameter $K(mm^2/s^2)$ represents the viscometer constant and is previously determined by using the Standard Liquids for Calibrating Viscometers with a known kinematic viscosity. In the case of a viscometer used for a sample having a similar viscosity to water, water itself can be used as are reference standard liquid for the calibration. The kinematic viscosity of water is 1.0038 mm^2/s at 20 °C. In the cases of viscometers used for sample liquids having a slightly higher viscosity, the Standard Liquids for Calibrating Viscometers are used for the calibration.

The intrinsic viscosity, $[\eta](mL/g)$, of a polymer solution is obtained by plotting the relation of viscosity versus concentration and extrapolating the obtained straight line to zero concentration. Intrinsic viscosity shows the degree of molecular expansion of a polymer substance in a given solvent and is also a measure of the average molecular mass of the polymer substance.
The down flowing time, t(s), for a test solution, whose concentrationis c(g/100 mL), and t_0 (s) for the solvent used for dissolution of the sample, are measured by using the same viscometer, and then the intrinsic viscosity of a given polymer substance, $[\eta]$, is calculated according to the following equation:

$$[\eta] = \lim_{c \to 0} \frac{\ln \frac{t}{t_0}}{c} \quad \text{or} \quad [\eta] = \lim_{c \to 0} \frac{(\frac{t}{t_0}) - 1}{c}$$

When the concentration dependency of $(\ln t / t_0) / c$ is not large, the value of $(\ln t / t_0) / c$ at a concentration directed in the respective monograph can be assumed to be the intrinsic viscosity for a given substance.

Unless otherwise specified, the viscosity of a test solution is measured with the following apparatus and procedure.

Apparatus

For measurements of the kinematic viscosity in the range of 1 to 100000 mm^2/s ,the Ubbelohde-type viscometer illustrated in Fig. 1 is used. The approximate relations between kinematic viscosity ranges and inside diameters of the capillary tubes suitable for the measurement for various liquids with different kinematic viscosities, are given in the attached table. Although the inside diameter of the capillary tube needs not be exactly the same as shown in the table, a viscometer is selected with which the down flowing time, *t(s)*, shall be between 200s and 1000 s.

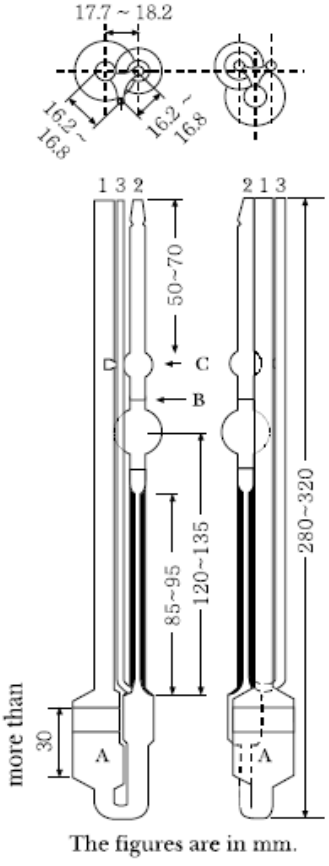


Fig 1. Ubbelohde type capillary tube viscometer

Procedure

Place a sample liquid in a viscometer from one end of tube 1, and adjust the meniscus of the liquid column to be at a level between the two marked lines of bulb A. Place the viscometer vertically in a thermostat-equipped bath maintained at a specified temperature within ±0.1 °C fully immersing bulb C, and let stand for 20 minutes to allow the test solution to come to the specified temperature. Close tube 3 with a finger and pull the test solution up to the middle part of bulb C by gentle suction from the top of tube 2, taking care not to introduce any bubbles into tube 2. Stop the suction, open the end of tube 3, and immediately close the end of tube 2. After the test solution below the bottom of the capillary has flowed down, open the end of tube 2 and record the time, *t(s)*, required for the meniscus of the test solution to pass from the upper marked line to the lower one of bulb *B*.

Determine the viscometer constant, *K*, previously, using the Standard Liquids for Calibrating Viscometers under the same conditions. But the temperature at which the calibration is performed, need not be the same as that for measuring sample viscosity.

Viscometer constant K	Inner 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

A rotational viscometer is usually used for measuring the viscosity of Newtonian or non-Newtonian liquids. The measuring principle of a rotational viscometer generally consists in the detection and determination of the force acting on a rotor (torque), when it rotates at a constant angular velocity in a liquid. The torque acting on the rotor surface is detected in terms of the torsion of a spring of the viscometer and the viscosity of the sample can be calculated from the scale-indicated value, which corresponds to the degree of torsion.

The viscosity of a sample liquid is measured with the following apparatus and procedure.

Apparatus

Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

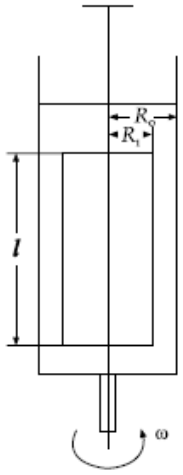


Figure 2a. Coaxial double cylinder type rotational viscometer.

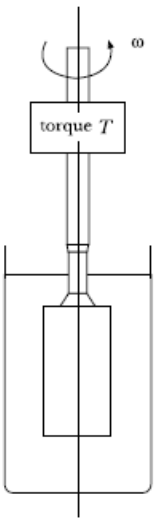


Figure 2b. Single cylinder type rotational viscometer

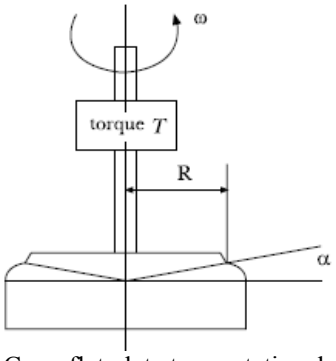


Fig 2c. Cone-flat plate type rotational viscometer

(1) *Coaxial double cylinder type rotational viscometer*

In the coaxial double cylinder type rotational viscometer, viscosity is determined by filling a liquid in the gap between the inner and outer cylinders, which share the

same central axis and rotate separately, and by measuring the generated torque acting on one cylinder surface when the other cylinder is rotated at a given angular velocity or by measuring the angular velocity of one cylinder when the other is rotated at a given torque.
As shown in Fig. 2a, the inner cylinder is hung by a wire whose twist constant is designated as k. Half the outer diameter of the inner cylinder and inner diameter of the outer cylinder 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 the outer cylinder is made to rotate at a constant angular velocity, ω, the inner cylinder is also forced to rotate under the action of viscosity caused by the liquid rotational flow. Consequently, torque, T , is generated by rotation of the inner cylinder in the medium, and in the steady state, the torque is balanced by the torsion of the wire, as indicated by the degree of rotation, θ. Then, the relationship can be expressed by $T = k \cdot \theta$, and the viscosity of the liquid, η, is determined from the following equation by measuring the relationship between ω and θ. Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship expressed by the equation holds.

$$\eta = \frac{100T}{4\pi l\omega}\left[\frac{1}{R_i^2} - \frac{1}{R_o^2}\right]$$

η: Viscosity of sample (m·Pas),
π: Circumference/diameter ratio,
l: Length of the inner cylinder (cm),
ω: Angular velocity (rad/s),
T: Torque acting on cylinder surface (10^{-7}N·m),
R_i: 1/2 of outer diameter of the inner cylinder (cm),
R_o: 1/2 of inner diameter of the outer cylinder (cm).

(2) *Single cylinder type rotational viscometer* In the single cylinder type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder surface when the cylinder immersed in a solution is rotated at a given angular velocity. The apparatus illustrated in Fig. 2b is used. If the apparatus constant, K_B, is previously determined experimentally by using the Standard Liquids for Calibrating Viscometers, the viscosity of a sample liquid, η , can be obtained from the following equation.

$$\eta = K_B \cdot \frac{T}{\omega}$$

η: Viscosity of sample (mPa·s),
K_B: Apparatus constant of viscometer (rad/cm^3).
ω: Angular velocity (rad/s), and
T: Torque acting on cylinder surface (10^{-7}N·m).

(3) *Conc-flat plate type rotational viscometer* In the cone-flat plate type rotational viscometer, viscosity is determined by filling a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotating axis, and by measuring the torque or angular velocity of either the disc or the cone affected by the opposing counterpart cone or disc. The apparatus illustrated in Fig. 2c is used.

A liquid is introduced to fill the gap between a flat disk and a cone forming an angle, α. As either the flat disk or the cone is rotated at a constant angular velocity or a constant torque, the liquid flows rotationally and the viscosity is measured by determining the torque or the angular velocity acting on the opposing disk or cone in the steady state. The viscosity of the sample liquid, η,iscalculatedfromthefollowingequation.

$$\eta = 100 \times \frac{3\alpha}{2\pi R^3} \times \frac{T}{\omega}$$

η: Viscosity of sample (mPa·s),
π: Circumference/diameter ratio,
R: Radius of a flat disk (cm),
α: Angle between a flat disk and a cone (rad),
ω: Angular velocity (rad/s),
T:Torque acting on flat discorcone surface (10^{-7}N·m)

Procedure
Install the viscometer so that its rotating axis is perpendicular to the horizontal plane. Place a sufficient quantity of test liquid for measurement in the viscometer, and allow the measuring system to stand until a specified temperature has been attained, as directed in the monograph. Where it is desired to measure the viscosity with a precision of 1 percent, the temperature variation should be within ± 0.1 °C. After the temperature of the test liquid has reached the designated value, start operating the rotational viscometer. After the rotation of the viscometer has reached the steady state and the indicated value on the scale, which corresponds to the rotational frequency or the torque, has become constant, read the value on the scale. Then, calculate the viscosity, η, by using the equation appropriate to the type of viscometer being used. Determination of the apparatus constant should be conducted experimentally before hand by using the Standard Liquids for Calibrating Viscometers, and the validation of the apparatus and operating procedure shall also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of liquid with variation of the rotation velocity or torque from one measurement to another. From this series of viscosity measurements, the relationship between the apparent viscosity and the slip velocity of a non-Newtonian liquid, i.e., the flow characteristics of a non-Newtonian liquid, can be obtained.

Calibration of a rotational viscometer is conducted by using water and the Standard Liquids for Calibrating Viscometers. These standard liquids are used for the determination of the apparatus constant of the rotational viscometer. They are also used for periodic re-

calibration of the viscometer to confirm a specified precision.

62. Vitamin A Assay

The Vitamin A Assay is a method to determine vitamin A by ultraviolet absorption spectrophotometry in retinol acetate, retinol palmitate, vitamin A oil, cod liver oil, and other preparations. However, proper pretreatments are generally necessary depending on the kind of preparations or on the existence of substances which disturb the assay. One vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.3 μg of vitamin A (all-trans vitamin A alcohol).

Reagents

2-propanol and ether used in the assay meet the following requirements.

2-propanol: Read the absorbance as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: The absorbance at 300 nm is not more than 0.05, and the absorbance between 320 nm and 350 nm is not more than 0.01. If necessary, it should be purified by distillation.

Ether: Freshly distill, discarding the first and last 10 percent proportions.

Procedure

All procedures should be carried out quickly, care must be taken as far as possible to avoid exposure to air and to oxidizing agents, and light-resistant vessels are used.

Unless otherwise specified in the monograph, proceed by Method 1, but apply Method 2 when the assay conditions required for Method 1 are not available.

(1) Method 1 Weigh accurately about 0.5 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 250 mL. Dilute this solution with 2-propanol to make a solution having an absorbance of about 0.5 at 326 nm when it is determined as directed under the Ultraviolet-visible Spectrophotometry, and use this solution as the test solution. Determine the wavelength of the maximum absorption and the absorbance at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm, and calculate the relative extinction of each wavelength, taking the absorbance at 326 nm as 1.000. When the maximum absorption lies between 325 nm and 328 nm, and the relative extinction of each wavelength is with in the range of ± 0.030 of the values in the table, the potency of vitamin A in unit per g of the sample is calculated from the absorbance A at 326 nm.

$$\text{Units of vitamin A in 1 g} = E_{1cm}^{1\%}(326\,\text{mm}) \times 1900$$

$$E_{1\,\text{cm}}^{1\%}(326\ \text{nm}) = \frac{A}{W} \times \frac{V}{100}$$

V : Total volume(mL) of the test solution

W : Amount(g) of sample in VmL of the sample solution

1900 : Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

l (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

Perform the following test to identify retinol acetate and retinol palmitate

Identification Dissolve amounts or volumes, equivalent to 15000 vitamin A units, of the sample, Retinol Acetate RS and Retinol Palmitate RS in 5mL portions of petroleum ether separately, and use these solutions as the test solution and the standard solutions. Perform the test directed under the Thin-Layer Chromatography. Spot 5 μL each of the test solution and the standard solutions on a plate of silica gel for thin-layer chromatography, develop it to a distance of about 10 cm with a mixture of cyclohexane and ether (12 : 1) as the solvent, and air-dry the plate. Spray antimony(III) chloride TS on the plate, and identify by comparing the position of the principal blue-colored spots of the sample and the standards.

When the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the relative extinction is not within the range of ± 0.030 of values the table, proceed with the test by Method 2.

(2) Method 2 Unless otherwise specified, weigh accurately a sample containing not less than 500 units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30mL of aldehyde-free ethanol and 1mL of a solution of pyrogallol in ethanol (1 in 10). Then add 3mL of a solution of potassium hydroxide (9 in 10), attach a reflux condenser, and heat on a water-bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30mL of water, transfer to a separator A, wash the flak with 10mL of water and then 40mL of ether, transfer the washings to the separator A, shake well, and allow to stand. Transfer the water layer in separator A to a separator B, wash the flask with 30mL of ether, transfer the washing to separator B, and extract by shaking. Transfer the water layer to a flask, add ether layer to separator A, transfer the water layer to separator B, add 30mL of ether, and extract by shaking. Add the ether layer to separator A. Add 10mL of water, allow to stand calmly after gentle turning upside down 2 to 3 times, and remove the water layer. Further wash with three 50-mL volumes of water until the washing gives no pink color with phenolphthalein TS,

and allow to stand for 10 minutes. Remove the remaining water as far as possible, transfer the ether to an Erlenmeyer flask, wash the separator with two 10-mL volumes of ether, add the washings to the flask, add 5 g of anhydrous sodium sulfate to the ether solution, mix by shaking, and transfer the ether to a round-bottomed flask by decantation. Wash the remaining sodium sulfate with two or more 10-mL volumes of ether, and transfer the washings to the flask.

Evaporate the ether solution in a water bath at 45 °C with swirling of the flask, using an aspirator, to about 1mL, immediately add sufficient 2-propanol to make a solution containing 6 to 10 vitamin A units permL, and designate the solution as the test solution.

Read the absorbances, A_1 at 310nm, A_2 at 325 nm, and A_3 at 334 nm, of the sample solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Units of vitamin A in 1 g of the sample} = E^{1\%}_{1\,\mathrm{cm}}\ (325\ \mathrm{nm}) \times 1830$$

$$E^{1\%}_{1\,\mathrm{cm}}\ (325\ \mathrm{nm}) = \frac{A_2}{W} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f: Correction factor

V: Total volume(mL) of the test solution

W: Amount(g) of sample in VmL of the test solution

1830: Conversion factor from specific absorbance of retinol alcohol to IU (Unit/g)

63. Water Determination (Karl Fischer Method)

Water Determination (Karl Fischer Method) is a method to determine water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine. The reaction proceeds in the manner shown in the following equation:

$$I_2 + SO_2 + 3C_5H_5N + CH_3OH + H_2O \rightarrow 2(C_5H_5N^+H)^- OSO_2OCH_3$$

In this measurement there are two methods different in iodine-providing principle: one is the volumetric titration method and the other, the coulometric titration method. In the former, iodine is previously dissolved in a reagent for water determination, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water. In the latter, iodine is produced by electrolysis of Karl Fischer reagent containing iodide ion. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be determined by measuring the quantity of electricity which is required for the production of iodine during the titration.

$$2I^- \rightarrow I_2 + 2e^-$$

1. Volumetric titration

1) *Apparatus*

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

The Karl Fischer TS is extremely hygroscopic, so the apparatus should be designed to be protected from atmospheric moisture. Desiccants such as silica gel or calcium chloride for water determination are used for moisture protection.

2) *Reagents*

(1) *Chloroformforwaterdetermination* - To 1000 mL of chloroform, add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg permL.

(2) *Method for water determination* - To 1000 mL of methanol, add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg permL.

(3) *Propylene carbonate for water determination* - To 1000 mL of propylene carbonate, add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protecting from moisture .The water content should not be more than 0.3 mg permL.

(4) *Diethylene glycol monoethyl ether for water determination* - To 1000 mL of diethylene glycol monoethyl ether, add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve the diethylene glycol monoethy ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg permL.

(5) *Pyridine for water determination* - Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine should not be more than 1 mg permL.

(6) *Imidazole for water determination* - Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg permL.

(7) *2-Methylaminopyridine for water determination* - Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyridine should not be more than 1 mg permL.

3) *Preparation of test solutions and standard solutions*

(1) *Karl Fischer TS for water determination* The Karl Fischer TS is preserved in a cold place, protecting it from light and moisture.

① Preparation Prepare according to the following method(i), (ii) or (iii). Additives may be added for the purpose of improving the stability of other performances if it is confirmed that they give almost the same results as those obtained from the specified method.

(i) *Preparation 1*

Dissolve 63 g of iodine in 100mL of pyridine for water determination, cool the solution in ice-bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500mL by adding chloroform for water determination or methanol for water determination, and allow to stand for more than 24 hours before use.

(ii) *Preparation 2*

Dissolve 102 g of imidazole for water determination in 350mL of diethylene glycol monoethyl ether for water determination, cool the solution in ice-bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 64 g, keeping the temperature between 25 °C and 30 °C. Then dissolve 50 g of iodine in this solution, and allow to stand for more than 24 hours before use.

(iii) *Preparation 3*

Pass dried sulfur dioxide gas through 220mL of propylene carbonate until the mass increase of the solution reaches 32 g. To this solution, cooled in ice bath, add 180 mL of propylene carbonate or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for water determination is dissolved. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

② Standardization Perform the standardization of Karl Fischer TS before use. According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the solvent with a Karl Fischer TS to make the content of the flask anhydrous. Then, add quickly about 30 mg of water weighed accurately to the solution in the flask, and titrate the water dissolved in the solvent with a Karl Fischer TS to the endpoint, under vigorous stirring. Calculate the water equivalence factor, f(mg/mL), corresponding to the amount of water(H_2O) in mg per 1mL of the test solution by using the following equation:

$$f\,(\mathrm{mg/mL}) = \frac{\text{Amount of water } (H_2O) \text{ taken (mg)}}{\text{Volume of Karl Fischer TS consumed for titration of water } (H_2O)\,(\mathrm{mL})}$$

(2) *Standard water-methanol solution* Standard water-methanol solution is preserved in a cold place, protecting it from light and moisture.

① Preparation - Take 500 mL of methanol for water determination in a dried 1000 mL volumetric flask, add 2.0 mL of water, and adjust with the methanol to make 1000 mL.

② Standardization - Perform the standardization of this solution, followed by the procedure for Karl Fischer TS. According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Then, add exactly 10mL of Karl Fischer TS to this solution I the flask, and titrate it with the prepared standard water-methanol solution to the end point. Calculate the water concentration in the standard water-methanol solution, *f'*(mg/mL),by using the following equation:

$$f'\,(\mathrm{mg/mL}) = \frac{f\,(\mathrm{mg/mL}) \times 10\,(\mathrm{mL})}{\text{Volume of the standard water - methanol solution consumed for titration (mL)}}$$

4) *Procedure*

As a rule, the titration of water with a Karl Fischer TS should be performed at the same temperature as that at which the standardization was done, with protection from moisture. The apparatus is equipped with a variable resistor in the circuit, and this resistor is manipulated so as to maintain a constant voltage (mV) between two platinum electrodes immersed in the solution to be titrated. The variable current (μA) can be measured (Amperometric titration at constant voltage). During titration with Karl Fischer TS, the current in the circuit varies noticeably, but returns to the original values within several seconds. At the end of a titration, the current stops changing and persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

Otherwise, the manipulation of the resistor serves to pass a definite current between platinum electrodes. The variable potential (mV) can be measured (Potentiometric titration at constant current). With the progress of titration of water with a Karl Fischer TS, the value indicated by the potentiometer in the circuit decreases suddenly form a polarization state of several hundred (mV) to the non-polarization state, but it returns to the original value within several seconds. At the end of titration, the non-polarization state persists for certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

In the case of back titration, when the amperometric titration method is used at constant voltage, the needle of microammeter is out of scale during excessive presence of Karl Fischer TS, and it returns rapidly to the original position when the titration system has reached the end point. In the case of potentiometric titration method at constant current in the back titration mode, the needle of the millivoltmeter is at the original position during excessive presence of Karl Fischer TS. Finally a definite voltage is indicated when the titration system has reached the end point.

Unless otherwise specified, the titration of water with Karl Fischer TS can be performed by either one of the following methods. Usually, the end point of the titration can be observed more clearly in the back titration method, compared with the direct titration method.

(1) *Direct titration*

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a quantity of sample specimen containing 5 to 30 mg of water, transfer it quickly into the titration flask, dissolve by stirring, and titrate the solution to be examined with Karl Fischer TS to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount of the sample containing 5 to 30 mg of water, and transfer it quickly into the titration vessel, stir the mixture for 5 to 30 minutes, protecting it from moisture, and perform a titration under vigorous stirring. Alternatively, in the case of a sample specimen which is insoluble in the solvent for water determination or which interferes with the Karl Fischer reaction, water in the sample can be removed by heating under a stream of nitrogen gas, and introduced into the titration vessel by using a water evaporation technique.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water } (H_2O)\,\% = \frac{V\ (\text{mL}) \times f\ (\text{mg/mL})}{\text{Amount of sample (mg)}} \times 100$$

V : Volume of Karl Fischer TS consumed for titration

(2) *Backtitration*

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in the dried titration vessel, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a suitable quantity of sample specimen having 5 to 30 mg of water, transfer the sample quickly into the titration vessel, dissolve it in the solution by stirring, add an excessive and definite volume of Karl Fischer TS, and then titrate the solution with the standard water-methanol solution to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount accurately, transfer it quickly into the titration vessel, and add an excessive and definite volume of Karl Fischer TS. After stirring for 5 to 30 minutes, with protection from moisture, perform the titration under vigorous stirring.

$$\text{Water } (H_2O)\,\% = \frac{\{V_0\ (\text{mL}) \times f\ (\text{mg/mL}) - V\ (\text{mL}) \times f'\ (\text{mg/mL})\}}{\text{Amount of sample (mg)}} \times 100$$

V_0 : Volume of Karl Fischer TS added

V : Volume of standard water-methanol solution consumed for titration

2. Coulometric titration

1) *Apparatus*

Usually, the apparatus is comprised of a titration flask equipped with an electrolytic cell for iodine production, a stirrer, and a potentiometric titration system at constant current. The iodine production system is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination.

Both electrodes are usually made of platinum-mesh. Because both the anolyte and the catholyte solutions for water determination are strongly hygroscopic, the titration system should be protected from atmospheric moisture. For this purpose, silica gel or calcium chloride for water determination can be used.

2) *Preparation of anolyte and catholyte solutions for water determination*

Electrolyte solutions shall consist of an anolyte solution and a catholyte solution, the preparations of which are described below.

Preparation- Any of methods (1), (2), and (3) can be used for the preparation of the electrolytes for coulometric titration.

(1) *Preparation 1*

Anolyte for water determination - Dissolve 102 g of imidazole for water determination in 900mL of methanol for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through the solution, which is kept below 30 °C. When the mass increase of the solution has reached 64 g, the gas flow is stopped and 12 g of iodine is dissolved by stirring. Then drop a suitable amount of water into the solution until the color of liquid is changed from brown to yellow, and add methanol for water determination to make up 1000mL.

Catholyte for water determination - Dissolve 24 g of diethanolamine hydrochloride in 100mL of methanol for water determination.

(2) *Preparation 2*

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 dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 25 g, the gas flow is stopped. Add 50mL of propylene carbonate, and dissolve 6 g of iodine in the solution. Then make up the solution to 500 mL by addition of methanol for water determination and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination - Dissolve 30 g of choline hydrochloride into methanol for water determination and adjust the volume to 100mL by adding the methanol.

(3) *Preparation 3*

Anolyte for water determination - Dissolve 100 g of diethanolamine in 900 mL of methanol for water determination or a mixture of methanol and chloroform for water determination (3:1), and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 64 g, the gas flow is stopped. Dissolve 20 g of iodine in the solution, and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination - Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitromethane (4:1).

3) *Procedure*

Take a suitable volume of an anolyte for water determination in the titration vessel, immerse in this solution a pair of platinum electrodes for potentiometric titration at constant current. Then immerse the iodine production system filled with a catholyte for water determination in the anolyte solution. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next take an accurately weighed amount of a sample specimen containing 0.2 to 5 mg of water, add it quickly to the vessel, dissolve by stirring, and perform the titration to the end point under vigorous stirring.

When a sample specimen cannot be dissolved in the anolyte, powder it quickly, and add an accurately weighed amount of the sample estimated to contain 0.2 to 5 mg of water to the vessel. After stirring the mixture for 5 to 30 minutes, with protection from atmospheric moisture, perform the titration under vigorous stirring. Alternatively, in the case of an insoluble solid or a sample containing a component which interferes with the Karl Fischer reaction, water in the sample can be removed by heating, and carried by a nitrogen gas flow into the titration vessel, by using a water evaporation technique.

Determine the quantity of electricity (C) [= electric current (A) × time (s)] required for the production of iodine during the titration, and calculate the water content (%) in the sample specimen by use of the following equation.

Through the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water } (H_2O)\ \% = \frac{\text{Quantity of electricity required for iodine production (C)}}{10.72\,(\text{C/mg}) \times \text{Amount of sample (mg)}} \times 100$$

10.72: quantity of electricity corresponding to 1 mg of water (C/mg)

64. X-Ray Powder Diffraction Method

X-Ray Powder Diffraction Method is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam.

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially 3 types of information can be derived from a powder diffraction pattern: angular position of diffraction lines (depending on geometry and size of the unit cell); intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample); and diffraction line profiles (depending on instrumental resolution, crystallite size, strain and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (for example, identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions(1) can also be made. The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually non-destructive in nature (specimen preparation is usually limited to grinding to ensure a randomly oriented sample). XRPD investigations can also be carried out under in situ conditions on specimens exposed to non-ambient conditions, such as low or high temperature and humidity.

1. Principle

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on the atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive

when the path difference between 2 diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see Fig. 1).

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

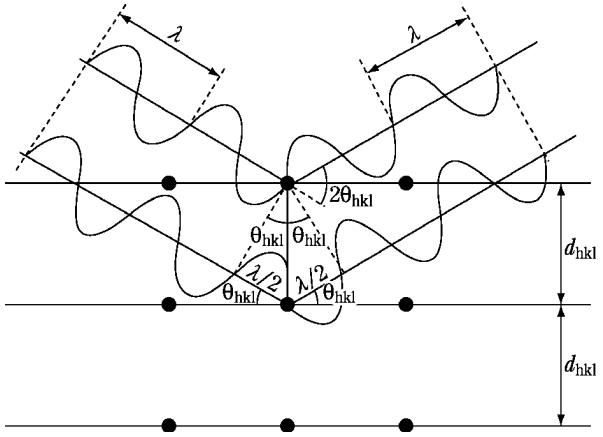


Fig. 1. Diffraction of X-rays by a crystal according to Bragg's law

The wavelength λ of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or d_{hkl} (also called `d-spacings'). θ_{hkl} is the angle between the incident ray and the family of lattice planes, and $\sin\theta_{hkl}$ is inversely proportional to the distance between successive crystal planes or d-spacings.

The direction and spacing of the planes with reference to the unit cell axes 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 cell axes. The unit cell dimensions are given by the spacings, *a*, *b* and *c* and the angles between them, α, β and v. The interplanar spacing for a specified set of parallel *hkl* planes is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the *d* values for the related families of planes, *nh*, *nk*, *nl* are diminished by the factor 1/n (n being an integer: 2,3,4, etc.). Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with it (for a specific wavelength λ).

A powder specimen is assumed to be polycrystalline so that at any angle θ_{hkl}l there are always crystallites in an orientation allowing diffraction according to Bragg's law.[2] For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as `lines', `reflections' or `Bragg reflections') are characteristic of the crystal lattice (d-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles on the perfection and extent of the crystal lattice. Under these conditions the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion and structural imperfections, as well as from instrument characteristics. The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity and Lorentz factor. The main characteristics of diffraction line profiles are 2θ position, peak height, peak area and shape (characterized by, for example, peak width or asymmetry, analytical function, empirical representation). An example of the type of powder patterns obtained for 5 different solid phases of a substance are shown in Fig. 2.

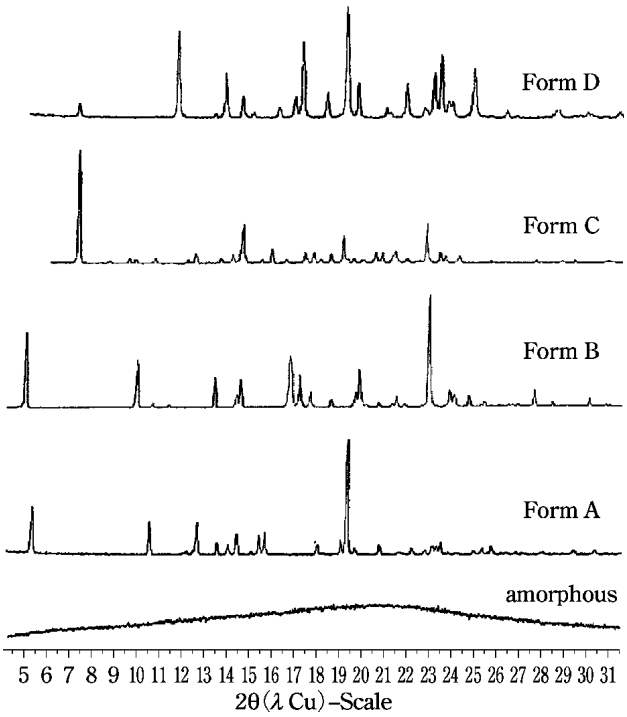


Fig. 2. X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities are normalized)

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more-or-less uniform background, upon which the peaks are superimposed. Besides specimen preparation, other factors contribute to the background, for instance the sample holder, diffuse scattering from air and equipment, other instrumental parameters such as detector noise, general radiation from the X-ray tube, etc. The peak to background ratio can be increased by minimizing background and by choosing prolonged exposure times.

2. Instrument

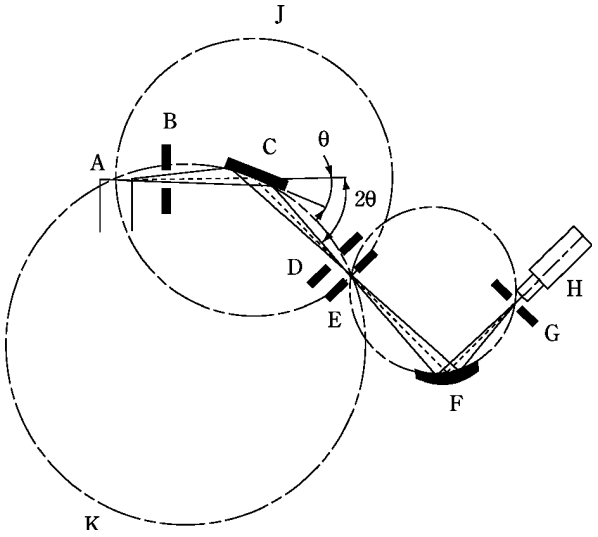
2.1. Instrument set-up

X-ray diffraction experiments are usually performed using powder diffractometers or powder cameras. A powder diffractometer generally comprises 5 main parts: an X-ray source; incident beam optics, which may perform monochromatization, filtering, collimation and/or focusing of the beam; a goniometer; diffraction beam optics, which may perform monochromatization, filtering, collimation and focusing or parallelising of the beam; and a detector. Data collection and data processing systems are also re-

quired and are generally included in current diffraction measurement equipment.

Depending on the type of analysis to be performed (phase identification, quantitative analysis, lattice parameters determination, etc.), different XRPD instrument configurations and performance levels are required. The simplest instruments used to measure powder patterns are powder cameras. Replacement of photographic film as the detection method by photon detectors has led to the design of diffractometers in which the geometric arrangement of the optics is not truly focusing but parafocusing, such as in the Bragg-Brentano geometry. The Bragg-Brentano parafocusing configuration is currently the most widely used and is therefore briefly described here.

A given instrument may provide a horizontal or vertical $\theta/2\theta$ geometry or a vertical θ/θ geometry. For both geometries, the incident X-ray beam forms an angle θ with the specimen surface plane and the diffracted X-ray beam forms an angle 2θ with the direction of the incident X-ray beam (an angle θ with the specimen surface plane). The basic geometric arrangement represented in Fig. 3. The divergent beam of radiation from the X-ray tube (the so-called `primary beam') passes through the parallel plate collimators and a divergence slit assembly and illuminates the flat surface of the specimen. All the rays diffracted by suitably oriented crystallites in the specimen at an angle 2θ converge to a line at the receiving slit. A second set of parallel plate collimators and a scatter slit may be placed either behind or before the receiving slit. The axes of the line focus and of the receiving slit are at equal distances from the axis of the goniometer. The X-ray quanta are counted by a radiation detector, usually a scintillation counter, a sealed-gas proportional counter, or a position-sensitive solid-state detector such as imaging plate or CCD detector. The receiving slit assembly 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 at half the rotational speed, in $\theta/2\theta$ motion. The surface of the specimen thus remains tangential to the focusing circle. The parallel plate collimator limits the axial divergence of the beam and hence partially controls the shape of the diffracted line profile.



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

Fig. 3. Geometric arrangement of the Bragg-Brentano parafocusing geometry

A diffractometer may also be used in transmission mode. The advantage with this technology is to lessen the effects due to preferred orientation. A capillary of about 0.5 . 2 mm thickness can also be used for small sample amounts.

2.2. X-ray radiation

In the laboratory, X-rays are obtained by bombarding a metal anode with electrons emitted by the thermionic effect and accelerated in a strong electric field (using a high-voltage generator). Most of the kinetic energy of the electrons is converted to heat, which limits the powder of the tubes and requires efficient anode cooling. A 20- to 30-fold increase in brilliance can be obtained using rotating anodes and by using X-ray optics. Alternatively, X-ray photons may be produced in a large-scale facility (synchrotron).

The spectrum emitted by an X-ray tube operating at sufficient voltage consists of a continuous background of polychromatic radiation and additional characteristic radiation that depends on the type of anode. Only this characteristic radiation is used in X-ray diffraction experiments. The principal radiation sources utilized for X-ray diffraction are vacuum tubes utilizing copper, molybdenum, iron, cobalt or chromium as anodes; copper, molybdenum or cobalt X-rays are employed most commonly for organic substances (the use of cobalt anodes can be especially preferred to separate

distinct X-ray lines). The choice of radiation to be used depends on the absorption characteristics of the specimen and possible fluorescence by atoms present in the specimen. The wavelengths used in powder diffraction generally correspond to the K_α radiation from the anode. Consequently, it is advantageous to make the X-ray beam `monochromatic' by eliminating all the other components of the emission spectrum. This can be partly obtained 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 tube.

Such a filter is usually inserted between the X-ray tube and the specimen. Another, more-and-more-commonly used way to obtain a monochromatic X-ray beam is via a large monochromator crystal (usually referred to as a `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 $K_{\alpha1}$ and $K_{\alpha2}$ radiations by using a specialized monochromator. Unfortunately, the gain in getting a monochromatic beam by using a filter or a monochromator is counteracted by a loss in intensity. Another way of separating K_α and K_β wavelengths is by using curved X-rays mirrors that can simultaneously monochromate and focus or parallelize the X-ray beam.

2.3. Radiation protection

Exposure of any part of the human body to X-rays can be injurious to health. It is therefore essential that whenever X-ray equipment is used, adequate precautions are taken to protect the operator and any other person in the vicinity. Recommended practice for radiation protection as well as limits for the levels of X-radiation exposure are those established by national legislation in each country. If there are no official regulations or recommendations in a country, the latest recommendations of the International Commission on Radiological Protection should be applied.

3. Specimen preparation and mounting

The preparation of the powdered material and mounting of the specimen in a suitable holder are critical steps in many analytical methods, and are particularly so for X-ray powder diffraction analysis, since they can greatly affect the quality of the data to be collected.[3] The main sources of error due to specimen preparation and mounting are briefly discussed here for instruments in Bragg-Brentano parafocusing geometry.

3.1. Specimen preparation

In general, the morphology of many crystalline particles tends to give a specimen that exhibits some degree of 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. Preferred orientation in the specimen influences the intensities of various reflections, so that some are more intense and others are less intense, compared to what would be expected from a completely random specimen. Several techniques can be employed to improve randomness in the orientation of crystallites (and therefore to minimize preferred orientation), but further reduction of particle size is often the best and simplest approach. The optimum number of crystallites depends on the diffractometer geometry, the required resolution and the specimen attenuation of the X-ray beam. In some cases, particle sizes as large as 50 μm will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately 0.5 μm) may cause line broadening and significant changes to the sample itself such as:

(i) specimen contamination by particles abraded from the milling instruments (mortar, pestle, balls, etc.);
(ii) reduced degree of crystallinity;
(iii) solid-state transition to another polymorph;
(iv) chemical decomposition;
(v) introduction of internal stress;
(vi) solid-state reactions.

Therefore, it is advisable to compare the diffraction pattern of the non-ground specimen with that corresponding to a specimen of smaller particle size (e.g. a milled specimen). If the X-ray powder diffraction pattern obtained is of adequate quality considering its intended use, then grinding may not be required. It should be noted that if a sample contains more than one phase and if sieving is used to isolate particle to a specific size, the initial composition may be altered.

4. Control of the instrument performance

Goniometers and the corresponding incident and diffracted X-ray beam optics have many mechanical parts that need adjustment. The degree of alignment or misalignment directly influences the quality of the results of an XRPD investigation. Therefore, the different components of the diffractometer must be carefully adjusted (optical and mechanical systems, etc.) to adequately minimize systematic errors, while optimizing the intensities received by the detector. The search for maximum intensity and maximum resolution is always antagonistic when aligning a diffractometer. Hence, the best compromise must be sought whilst performing the alignment procedure. There are many different configurations and each supplier's equipment requires specific alignment procedures.

The overall diffractometer performance must be tested and monitored periodically using suitable certified reference materials. Depending on the type of analysis, other well-defined reference materials may also be employed, although the use of certified reference materials is preferred.

5. Qualitative phase analysis (Identification of phases)

The identification of the phase composition of an unknown sample by XRPD is usually based on the visual or computer-assisted comparison of a portion of its X-ray diffraction powder pattern to the experimental or calculated pattern of a reference material. Ideally,

these reference patterns are collected on well-characterized single-phase specimens. This approach makes it possible in most cases to identify a crystalline substance by its 2θ diffraction angles or d-spacings and by its relative intensities. The computer-aided comparison of the diffraction pattern of the unknown sample to the comparison data can be based either on a more-or-less extended 2θ-range of the whole diffraction pattern or on a set of reduced data derived from the pattern. For example, the list of d-spacings and normalized intensities I_{norm}, a so-called (d, I_{norm})-list extracted from the pattern, is the crystallographic fingerprint of the material, and can be compared to (d, I_{norm})-lists of single-phase samples complied in databases.

For most organic crystals, when using Cu K_α radiation, it is appropriate to record the diffraction pattern in a 2θ-range from as near 0° as possible to at least 40°. The agreement in the 2θ-diffraction angles between specimen and reference is within 0.2° for the same crystal form, while relative intensities between specimen and reference may vary considerably due to preferred orientation effects. By their very nature, variable hydrates and solvates are recognized to have varying unit cell dimensions and as such shifting occurs in peak positions of the measured XRPD patterns for these materials. In these unique materials, variance in 2-θ positions of greater than 0.2° is not unexpected. As such, peak position variances such as 0.2° are not applicable to these materials. For other types of samples (e.g. inorganic salts), it may be necessary to extend the 2θ-region scanned to well beyond 4°. It is generally sufficient to scan past the 10 strongest reflections identified in single phase X-ray powder diffraction database files.

It is sometimes difficult or even impossible to identify phases in the following cases:

(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 per cent m/m);

(iii) pronounced preferred orientation effects;

(iv) the phase has not been filed in the database used;

(v) formation of solid solutions;

(vi) presence of disordered structures that alter the unit cell;

(vii) the specimen comprises too many phases;

(viii) presence of lattice deformations;

(ix) structural similarity of different phases.

6. Quantitative phase analysis

If the sample under investigation is a mixture of 2 or more known phases, of which not more than 1 is amorphous, the percentage (by volume or by mass) of each crystalline phase and of the amorphous phase can, in many cases, be determined. Quantitative phase analysis can be based on the integrated intensities, on the peak heights of several individual diffraction lines,[4] or on the full pattern. These integrated intensities, peak heights or full-pattern data points are compared to the corresponding values of reference materials. These reference materials shall be single-phase or a mixture of known phases. The difficulties encountered during quantitative analysis are due to specimen preparation (the accuracy and precision of the results require in particular homogeneity of all phases and a suitable particle size distribution in each phase) and to matrix effects. In favorable cases, amounts of crystalline phases as small as 10 per cent may be determined in solid matrices.

6.1. Polymorphic samples

For a sample composed 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 is derived by measuring the intensity ratio between the 2 phases, knowing the value of the constant K.

K is the ratio of the absolute intensities of the 2 pure polymorphic phases I_{oa}/I_{ob}. Its value can be determined by measuring standard samples.

6.2. Methods using a standard

The most commonly used methods for quantitative analysis are:

- the `external standard method';
- the `internal standard method';
- the `spiking method' (often also called the `standard addition method').

The `external standard method' is the most general method and consists of comparing the X-ray diffraction pattern of the mixture, or the respective line intensities, with those measured in a reference mixture or with the theoretical intensities of a structural model, if it is fully known.

To limit errors due to matrix effects, an internal reference material with crystallite size and X-ray absorption coefficient comparable to those of the components of the sample, and with a diffraction pattern that does not overlap at all that of the sample to be analyzed, can be used. A known quantity of this reference material is added to the sample to be analyzed and to each of the reference mixtures. Under these conditions, a linear relationship between line intensity and concentration exists. This application, called the `internal standard method', requires a precise measurement of diffraction intensities.

In the `spiking method' (or `standard addition method'), some of the pure phase a is added to the mixture containing the unknown concentration of a. Multiple additions are made to prepare an intensity-versus-concentration plot in which the negative x intercept is the concentration of the phase a in the original sample.

7. Estimate of the amorphous and crystalline fractions

In a mixture of crystalline and amorphous phases, the crystalline and amorphous fractions can be estimated in several ways. The choice of the method used depends on the nature of the sample:

(i) if the sample consists of crystalline fractions and an amorphous fraction of different chemical compositions, the amounts of each of the individual crystalline phases may be estimated using appropriate standard substances as described above; the amorphous fraction is then deduced indirectly by subtraction;

(ii) if the sample consists of one amorphous and one crystalline fraction, either as a 1-phase or a 2-phase mixture, with the same elemental composition, the amount of the crystalline phase (`the degree of crystallinity') can be estimated by measuring 3 areas of the diffractogram:

A = total area of the peaks arising from diffraction from the crystalline fraction of the sample:

B = total area below area A;

C = background area (due to air scattering, fluorescence, equipment, etc.)

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

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

It is noteworthy that this method does not yield absolute degree-of-crystallinity values and hence is generally used for comparative purposes only. More sophisticated methods are also available, such as the Ruland method.

8. Single crystal structure

In general, the determination of crystal structures is performed from X-ray diffraction data obtained using single crystals. However, crystal structure analysis of organic crystals is a challenging task, since the lattice parameters are comparatively large, the symmetry is low and the scattering properties are normally very low. For any given crystalline form of a substance, knowledge of the crystal structure allows the calculation of the corresponding XRPD pattern, thereby providing a `preferred-orientation-free' reference XRPD pattern, which may be used for phase identification.

(1) There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances such as: determination of crystal structures, refinement of crystal structures, determination of crystallographic purity of crystalline phases, characterization of crystallographic texture, etc. These applications are not described in this chapter.

(2) An `ideal' powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.

(3) Similarly, changes in the specimen can occur during data collection in the case of a non-equilibrium specimen (temperature, humidity).

(4) If the crystal structures of all components are known, the Rietveld method can be used to quantify them with good accuracy. If the crystal structures of the components care not known, the Pawley or least squares methods can be used.

65. Reference Standards; Reagents,Test Solutions; Standard Solutions for Volumetric Analysis; Standard Solutions;Matching Fluids for Color; Optical Filters for Wavelength and Transmission Rate Calibration; Measuring Instruments, Appliances; Sterilization and Aseptic Manipulation

Reference Standards are substances which are prepared to have definite purity or definite biological action, and are used when drugs are tested physically, chemically or biologically. Reference standards are used in biological and physicochemical tests.

Reagents are used in the tests defined in the Korean Pharmacopoeia. When designated as “Same as the namesake monograph in Part II”, the reagent conforms requirements in the corresponding monograph.

Test Solutions are prepared for the tests of the Korean Pharmacopoeia.

Standard Solutions for Volumetric Analysis are solutions of reagents of precisely known concentrations intended primarily for use in quantitative determinations.

Standard Solutions are solutions to be used as the bases for comparison in the tests of the Korean Pharmacopoeia.

Matching Fluids for Color are used as references for comparison of colors in the tests of the Korean Pharmacopoeia.

Measuring Instruments are instruments or machines used for the measurements in the tests of the Korean Pharmacopoeia.

1) Reference Standards

The Korean Pharmacopoeia Reference Standard are as follows:

Acanthoside D RS, Acarbose RS, Acebutolol Hydrochloride RS, Aceclofenac RS, Acetaldehyde RS, Acetaminophen RS, Acetylcholine Chloride RS, Acetylcysteine RS, Acetylspiramycin Ⅱ RS, Acetylspiramycin RS, Aconitine RS, Acrinol Hydrate RS, Acrinol RS, Acyclovir RS, Adenosine RS, Albendazole RS, Albiflorin RS, Alfacalcidol RS, Alfuzosin Hydrochloride RS, Arecoline Hydrobromide RS, Arginine Hydrochloride RS, Alimemazine Tartrate RS, Allantoin RS, Allopurinol RS, Almagate RS, Aloeemodin RS, Alprostadil RS, Amantadine Hydrochloride RS, Amidotrizoic Acid RS, Amikacin Sulfate RS, Aminobutanol RS, Aminocaproic Acid RS, 3-Aminopent-4-ene-1,1-Dicarboxylic Acid RS, Amitriptyline Hydrochloride RS, Amlodipine Besylate RS, Amoxicillin RS, Amphotericin B RS, Ampicillin RS, Ampicillin Sodium RS, Amygdalin RS, Anesaldehyde RS, Anetole RS, Anhdrous Caffeine RS, Anhydrous Lactose RS, Anthralin RS, Arbekacin Sulfate RS, Arctigenin RS, Arginine RS, Aristolokinic Acid RS, Arotinolol Hydrochloride RS, Ascorbic Acid RS, Aspirin RS, Aspoxicillin RS, Astromicin Sulfate RS, Atenolol RS, Atorvastatin Calcium RS, Atracurium Besylate RS, Atropine Sulfate Hydrate RS, Atropine Sulfate RS, Azathioprine RS, Azelastine Hydrochloride RS, Azithromycin RS, Aztreonam RS, Bacampicillin Hydrochloride RS, Bacitracin RS, Bacitracin Zinc RS, Baclofen RS, Baicalein RS, Baicalin RS, Bambuterol Hydrochloride RS, Bamethan Sulfate RS, Beclomethasone Propionate RS, Benserazide Hydrochloride RS, Benzalkonium Chloride RS, Benzbromarone RS, Benzethonium Chloride RS, Benzoic Acid RS, Benzydamine Hydrochloride RS, Benzyl Alcohol RS, Berberine Chloride Hydrate RS, Berberine Chloride RS, Berberine Tannate RS, Betahistine Mesilate RS, Betaine RS, Betamethasone Dipropionate RS, Betamethasone RS, Betamethasone Sodium Phosphate RS, Betamethasone Valerate RS, Betaxolol Hydrochloride RS, Bethanechol Chloride RS, Bezafibrate RS, Bifonazole RS, Bilirubine RS, Biperiden Hydrochloride RS, Bisacodyl RS, Bisdemethoxycurcumin RS, Bisoprolol Fumarate RS, Bleomycin A2 Hydrochloride RS, Bornyl Acetate RS, Bromazepam RS, Bromhexine Hydrochloride RS, Bromocriptine Mesilate RS, 8-Bromotheophylline RS, Bufalin RS, Bumetanide RS, Buspirone Hydrochloride RS, Busulfan RS, Butilscopolamin Bromide RS, Butyl Paraoxybenzoate RS, Caffeic Acid RS, Caffeine RS, Calcitriol RS, Calcium Gluconate Hydrate RS, Calcium p-Aminosalicylate Hydrate RS, Calcium Polystyrene Sulfonate RS, d-Camphor RS, dl-Camphor RS, Candesartan cilexitil RS, Capreomycin Sulfate RS, Capsaicin RS, Captopril RS, Carbamazepine RS, Carbazochrome Sodium Sulfonate Hydrate RS, L-Carbocisteine RS, Carboplatin RS, Cardamonin RS, Carisoprodol RS, Carmofur RS, Carteolol Hydrochloride RS, Carumonam Sodium RS, Carvedilol RS, Catalpol RS, Cathinone RS, Cefaclor RS, Cefadroxil RS, Cefalexin RS, Cefaloglycin RS, Cefalotin Sodium RS, Cefamandole Nafate RS, Cefamandole RS, Cefapirin Sodium RS, Cefatrizine Propylene Glycol RS, Cefazolin RS, Cefbuperazone Sodium RS, Cefcapene Pivoxil Hydrochloride RS, Cefixime RS, Cefmenoxime Hydrochloride RS, Cefmetazole RS, Cefmetazole Sodium RS, Cefminox Sodium RS, Cefodizime Sodium RS, Cefonicid Sodium RS, Cefoperazone RS, Cefotaxime Sodium RS, Cefotetane RS, Cefotiam Hexetil Hydrochloride RS, Cefotiam Hydrochloride RS, Cefoxitin RS, Cefpiramide RS, Cefpirome Sulfate RS, Cefpodoxime Proxetil RS, Cefprozil(E) Isomer RS, Cefprozil(Z) Isomer RS, Cefradine RS, Cefroxadine RS, Cefsulodin Sodium RS, Ceftazidime RS, Cefteram Pivoxil Mesitylene Sulfonate RS, Ceftibuten Hydrochloride RS, Ceftizoxime RS, Ceftizoxime Sodium RS, Ceftriaxone Sodium RS, Cefuroxime Axetil RS, Cefuroxime Sodium RS, Cellacefate RS, Cetirizine Hydrochloride RS, Cetraxate Hydrochloride RS, Chenodeoxycholic Acid RS, Chlorambucil RS, Chloramphenicol Palmitate RS, Chloramphenicol RS, Chloramphenicol Sodium Succinate RS, Chlordiazepoxide Hydrochloride RS, Chlordiazepoxide RS, Chlormadinone Acetate RS, Chlorogenic Acid RS, Chlorphenesin Carbamate RS, Chlorpheniramine Maleate RS, D-Chlorpheniramine Maleate RS, Chlorpromazine Hydrochloride RS, Chlorpropamide RS, Chlorzoxazone RS, Cholecalciferol RS, Chrysophanol RS, Ciclacillin RS, Cilazapril Hydrat RS, Cilostazol RS, Cimetidine Hydrochloride RS, Cimetidine RS, Cinchonidine RS, Cinchonine RS, Cineol RS, Cinnamic Acid RS, Cinnarizine RS, Cinobufagin RS, Ciprofloxacin Hydrochloride Hydrate RS, Cisplatin RS, Citric Acid Hydrate RS, Clarithromycin RS, Clavolanic Acid RS, Clebopride Malate RS, Clenbuterol Hydrochloride RS, Clindamycin Hydrochloride RS, Clindamycin Phosphate RS, Clinofibrate RS, Clobetasol Propionate RS, Clofibrate RS, Clomifene Citrate RS, Clomipramine Hydrochloride RS, Clonazepam RS, Clonidine Hydrochloride RS, Clopidogrel Bisulfate RS, Clotrimazole RS, Cloxacillin Sodium RS, Cocaine Hydrochloride RS, Codeine Phosphate Hydrate RS, Codeine Phosphate RS, Colchicine RS, Colistin Sodium Methanesulfonate RS, Coptisine RS, Cortisone Acetate RS, Croconazole Hydrochloride RS, Curcumin RS, Cyanamide RS, Cyanocobalamin RS, Cyanoguanidine RS, Cyclandelate RS, Cyclopentolate Hydrochloride RS, Cyclophosphamide Hydrate RS, Cyclophosphamide RS, Cycloserine RS, Cyproterone Acetate RS, Cysteine Hydrochloride RS, Cytarabine RS, Dactinomycin RS, Daidzin RS, Dantrolene Sodium Hydrate RS, Dapsone RS, Daunorubicin Hydrochloride RS, Decursin RS, Decursinol RS, Deferoxamine Mesilate RS, Demethoxycurcumin RS, Deslanoside RS, Desoximetasone RS, Desoxycorticosterone Acetate RS, Dexamethasone Phosphate RS, Dexamethasone RS, Dextromethorphan Hydrobromide RS, Diazepam RS, Dibekacin Sulfate RS, Dibucaine Hydrochloride RS, Dichlorodiamino Cyclohexane Platinum RS, Diclofenac Sodium RS, Diclofenamide RS, Dicloxacillin Sodium RS, Dicyclomine Hydrochloride

RS, Diethanolamine Fusidate RS, Diethylcarbamazine Citrate RS, Diethylene Glycol RS, Diflucortolone Valerate RS, Digitoxin RS, Digoxin RS, Dihydroergotamine Mesilate RS, Dilazep Hydrochloride RS, Diltiazem Hydrochloride RS, Dimenhydrinate RS, Dimercaprol RS, Dinoprostone RS, Diosmin RS, Diphenhydramine Hydrochloride RS, Dipyridamole RS, Dirithromycin RS, Disopyramide RS, Disulfiram RS, Dobutamine Hydrochloride RS, Domperidone Maleate RS, Domperidone RS, Dopamine Hydrochloride RS, Doxapram Hydrochloride Hydrate RS, Doxazocin Mesylate RS, Doxorubicin Hydrochloride RS, Doxycycline RS, Droperidole RS, Dydrogesterone RS, Ebastine RS, Edrophonium Chloride RS, Elcatonin RS, Emetine Hydrochloride RS, Emodin RS, Enalapril Maleate RS, Endotoxin RS, Enflurane RS, Enoxacin Hydrate RS, Enviomycin Sulfate RS, Ephedrine Hydrochloride RS, Ephedrine Sulfate RS, Epinephrine Hydrogen Tartrate RS, Epirubicin Hydrochloride RS, Ergocalciferol RS, Ergometrine Maleate RS, Ergotamine Tartrate RS, Erythromycin Estolate RS, Erythromycin Ethylsuccinate RS, Erythromycin Lactobionate RS, Erythromycin Oxime RS, Erythromycin RS, Erythromycin Stearate RS, Estradiol Benzoate RS, Estradiol RS, Estradiol Valerate RS, Estragole RS, Estriol RS, Estrone RS, Etacrynic Acid RS, Ethambutol Hydrochloride RS, Ethanol RS, Ethenzamide RS, Ethinylestradiol RS, Ethionamide RS, Ethosuximide RS, Ethyl Aminobenzoate RS, Ethyl Paraoxybenzoate RS, Ethylene Glycol RS, Etizolam RS, Etodolac RS, Etoposide RS, Eugenol RS, Evodiamine RS, Felodipine RS, Fenofibrate RS, Fenofibrate RS, Fenoprofen Calcium Hydrate RS, Fenoprofen Calcium RS, Fenoterol Hydrobromide RS, Fentanyl Citrate RS, Fenticonazole Nitrate RS, Ferrous Fumarate RS, Ferrous Gluconate II RS, Ferrous Sulfate RS, Finasteride RS, Flavin Adenine Dinucleotide Sodium RS, Flavoxate Hydrochloride RS, Flomoxef Sodium RS, Flomoxef Triethylammonium, Flubendazole RS, Flucloxacillin Sodium RS, Flucytosine RS, Fludrocortisone Acetate RS, Flumequine RS, Flunarizine Hydrochloride RS, Flunitrazepam RS, Fluocinolone Acetonide RS, Fluocinonide RS, 9-Fluorenylmethyl Chlorofromte RS, Fluorometholone RS, Fluoroquinolonic Acid RS, Fluorouracil RS, Fluoxetine Hydrochloride RS, Fluoxymesterone RS, Fluphenazine Enanthate RS, Flurazepam Hydrochloride RS, Flurazepam RS, Flurbiprofen RS, Fluticasone Propionate RS, Fluticasone Propionate Resolution Mixture RS(fluticasone propionate and fluticasone propionate related substance IV mixture), Fluticasone Propionate Resolution Mixture RS(fluticasone propionate and fluticasone propionate related substance II, III, IV mixture), Folic Acid RS, Folinate Calcium RS, Formononetin RS, Formoterol Fumarate Hydrate Dihydrate RS, Formoterol Fumarate Hydrate RS, Forsythoside A RS, Fosfomycin RS, Fosfomycin Phenethylammonium RS, Fructose RS, Fumaric Acid RS, Furosemide RS, Fursultiamine Hydrochloride RS, Gabexate Mesilate RS, Gallamine Triethiodide RS, Geniposide RS, Gentamicin Sulfate RS, Gentiopicroside RS, 6-Gingerol RS, Germacrone RS, Ginsenoside Rb1 RS, Ginsenoside Rg1 RS, Gitoxin RS, Glibenclamide RS, Gliclazide RS, Glimepiride RS, Gliquidone Sulfonamide RS, Glutamine RS, Glycerin RS, Glycine RS, Glycyrrhizic Acid RS, Gomisin A RS, Gomisin N RS, Gramicidin RS, Griseofulvin RS, Guaifenesin RS, Guanethidine Sulfate RS, Guanine RS, Haloperidol RS, Halothane RS, Heparin Sodium RS, Hesperidin RS, High Molecular Weight Urokinase RS, Honokiol RS, Human Chorionic Gonadotropin RS, Hydralazine Hydrochloride RS, Hydrochlorothiazide RS, Hydrocortisone Acetate RS, Hydrocortisone Butyrate RS, Hydrocortisone RS, Hydrocortisone Sodium Succinate RS, Hydrocortisone Succinate RS, Hydrocotarnine Hydrochloride RS, Hydroxypiperidine RS, Hydroxyprogesterone Caproate RS, Hymecromone RS, Hyosine Butylbromide RS, Hypromellulose Phthalate RS, Ibuprofen RS, Icariin RS, Idarubicin Hydrochloride RS, Idoxuridine RS, Ifenprodil Tartrate RS, Imidazole RS, Imipenem RS, Imipramine Hydrochloride RS, Imperatorin RS, Indapamide RS, Indigocarmine RS, Indometacin RS, Inositol RS, Insulin RS, Iodine RS, Iodixanol RS, Iodomethane RS, Iohexol RS, Iopamidol RS, Iopanoic Acid RS, Iopromide RS, Iospropyl Iodide RS, Iotalamic Acid RS, Ipratropium Bromide Hydrate RS, Isepamicin Sulfate RS, Isoconazole Nitrate RS, Isoflurane RS, Isoimperatorin RS, L-Isoleucine RS, Isoniazid RS, Isopropanol RS, Isoproterenol Hydrochloride RS, Isosorbide Dinitrate RS, Isosorbide RS, Isotretinoin RS, Isradipine RS, Josamycin RS, Kallidinogenase RS, Kanamycin Sulfate RS, Ketamine Hydrochloride RS, Ketoconazole RS, Ketoprofen RS, Ketorolac Tromethamine RS, Ketotifen Fumarate RS, Lacidipine RS, Lactitol Hydrate RS, Lactose Hydrate RS, Lactose RS, Lactulose RS, Lamivudine RS, Lansoprazole RS, Latamoxef Ammonium RS, Latamoxef Sodium RS, L-Leucine RS, Leonurine RS, Letrozole RS, Leucomycin A5 RS, Leucovorin Calcium RS, Levodopa RS, Levodropropizine RS, Levothyroxine Sodium Hydrate RS, Lidocaine RS, Lincomycin Hydrochloride Hydrate RS, Linderane RS, Liothyronine Sodium RS, Liquiritin RS, Liquirtigenin RS, Lisinopril Hydrate RS, Lithium Carbonate RS, Lithocholic Acid RS, Lysine Hydrochloride RS, Loganin RS, Loperamide Hydrochloride RS, Loracarbef RS, Losartan Potassium RS, Lovastatin RS, Loxoprofen RS, Loxoprofen Sodium Hydrate RS, Magnesium Aspartate Hydrate RS, Magnolol RS, Maltose Hydrate RS, D-Mannitol RS, Maprotiline Hydrochloride RS, Matrine RS, Mebendazole RS, Mebeverin Hydrochloride RS, Meclizine Hydrochloride RS, Meclocycline RS, Meclofenoxate Hydrochloride RS, Mecobalamin RS, Medroxyprogesterone Acetate RS, Mefenamic Acid RS, Megestrol Acetate RS, Melamine RS, Meloxicam RS, Melphalan RS, l-Menthol RS, Mepivacaine Hydrochloride RS, Meprobamate RS, Mequitazine RS, Mercaptopurine RS, Meropenem RS, Mesalazine RS, Mestranol RS, Metenolone Enanthate RS, Metformin Hydrochloride

RS, Methacycline Hydrochloride RS, Methamphetamine Hydrochloride RS, Methanol RS, DL-Methionine RS, Methocarbamol RS, Methotrexate RS, Methoxsalen RS, Methoxyethanol RS, Methyl Iodide RS, Methyl Paraoxybenzomate RS, Methyldopa Hydrate RS, Methyldopa RS, DL-Methylephedrine Hydrochloride RS, Methylergometrine Maleate RS, Methylparaben RS, Methylcarbamate RS, Methyldopa RS, Methylergometrine Maleate RS, Methylphenidate Hydrochloride RS, Methylprednisolone RS, Methylprednisolone Sodium Succinate RS, Methyltestosterone RS, Metoclopramide Hydrochloride RS, Metoclopramide RS, Metronidazole RS, Mexiletine Hydrochloride RS, Miconazole Nitrate RS, Midazolam RS, Midecamycin Acetate RS, Midecamycin RS, Minocycline Hydrochloride Hydrate RS, Mitomycin C RS, Mometasone Furoate RS, Morphine Hydrochloride Hydrate RS, Morphine Sulfate Hydrate RS, Morronisede RS, Mosapride Citrate RS, Mupirocin Lithium RS, Mupirocin RS, Nabumetone RS, Nalidixic Acid RS, Naloxone Hydrochloride RS, Naphazoline Nitrate RS, Naproxen RS, Naproxen Sodium RS, Naringin RS, Neomycin Sulfate RS, Neostigmine Bromide RS, Neostigmine Methylsulfate RS, Netilmicin Sulfate RS, Nicardipine Hydrochloride RS, Nicergoline RS, Nicorandil RS, Nicotinamide RS, Nicotinic Acid RS, Nifedipine RS, Nifuroxazide RS, Nimodipine RS, Nitrazepam RS, Nitrendipine RS, Nitroglycerin RS, Nizatidine RS, Nodakenin RS, Nordazepam RS, Norepinephrine Tartrate Hydrogen RS, Norepinephrine Tartrate RS, Norethisterone Acetate RS, Norethisterone RS, Norfloxacin RS, Norgestrel RS, Nortriptyline Hydrochloride RS, Noscapine Hydrochloride Hydrate RS, Noscapine RS, Nystatin RS, Ofloxacin RS, Oleanolic Acid RS, Omeprazole RS, Ondansetron Hydrochloride Hydrate RS, Ondansetron Hydrochloride RS, Orciprenaline Hydrochloride RS, Orciprenaline Sulfate RS, Orphenadrine Hydrochloride RS, Oxaliplatin RS, Oxapium Iodide RS, Oxaprozin RS, Oxethazaine RS, Oxprenolol Hydrochloride RS, Oxybuprocaine Hydrochloride RS, Oxycodone Hydrochloride Hydrate RS, Oxymatrine RS, Oxymetazoline Hydrochloride RS, Oxymetholone RS, Oxypeucedanin RS, Oxytetracycline RS, Oxytocin RS, Paclitaxel RS, Paeoniflorin RS, Paeonol RS, Palmatine RS, Palmitic Acid RS, Panipenem RS, Papaverine Hydrochloride RS, *p*-Amino Benzoylglutamic Acid RS, Paroxetine Hydrochloride Hydrate RS, Penbutolol Sulfate RS, Penicillin G Potassium RS, Penicillin G Sodium RS, Pentazocine RS, Pentobarbital RS, Pentoxifylline RS, Pentoxyverine Citrate RS, Perphenazine Maleate RS, Perphenazine RS, Pethidine Hydrochloride RS, Phenobarbital RS, Phenol RS, Phenolsulfonphthalein RS, L-Phenylalanine RS, Phenylephrine Hydrochloride RS, Phenytoin RS, Phloroglucinol RS, Phycion RS, Phytonadione RS, Pimaricin RS, Pimozide RS, Pindolol RS, Pinoresinol Diglucoside RS, Pipemidic Acid Hydrate RS, Piperacillin RS, Piperazine Citrate RS, Pirenzepine Hydrochloride Hydrate RS, Piperine RS, Piracetam RS, Pirarubicin RS, Piroxicam RS, Pivampicillin RS, Pivmecillinam Hydrochloride RS, Polydimethylsiloxane RS, Polymyxin B Sulfate RS, Poncirin RS, Potassium Gluconate RS, Potassium Guaiacolsulfonate RS, Potassium Iodide RS, Potassium Sucrose Octasulfate RS, Povidone RS, Pralidoxime Chloride RS, Pranoprofen RS, Pravastatin 1,1,3.3-Tetramethylbutylammonium RS, Pravastatin Sodium RS, Prednisolone Acetate RS, Prednisolone RS, Prednisolone Succinate RS, Primidone RS, Probenecid RS, Procainamide Hydrochloride RS, Procaine Hydrochloride RS, Procaterol Hydrochloride Hydrate RS, Prochlorperazine Maleate RS, Progesterone RS, Proglumide RS, Promethazine Hydrochloride RS, Propafenone Hydrochloride RS, Propafenone RS, Propofol RS, Propranolol Hydrochloride RS, Propyl Paraoxybenzoate RS, Propylthiouracil RS, Protamine Sulfate RS, Protirelin RS, Pseudoephedrine Hydrochloride RS, Puerarin RS, Pyrantel Pamoate RS, Pyrazinamide RS, Pyridostigmine Bromide RS, Pyridoxine Hydrochloride RS, Pyrvinium Pamoate RS, Quercetin Dihydrate RS, Quinine Sulfate Hydrate RS, Ramipril RS, Ranitidine Hydrochloride RS, Redibufogenin RS, Repaglinide RS, Reserpine RS, Retinol Acetate RS, Retinol Palmitate RS, Rhein RS, Riboflavin Butyrate RS, Riboflavin RS, Riboflavin Sodium Phosphate RS, Ribostamycin Sulfate RS, Rifabutin RS, Rifampicin RS, Rifamycin SV RS, Rifaximin RS, Risperidone RS, Rokitamycin, Roxithromycin RS, Rutecarpin RS, Rutin RS, Saccharated Pepsin RS, Saccharin Sodium Hydrate RS, Saikosaponin A RS, Saikosaponin D RS, Salbutamol Sulfate RS, Salicylic Acid RS, Salvianolic Acid RS, Sarpogrelate Hydrochloride RS, Sarsasapogenin RS, Schisandrin RS, Scopolamine Butylbromide RS, Selegiline Hydrochloride RS, Sennoside A RS, Sennoside B RS, Silver Sulfadiazine RS, Simethicone RS, Simvastatin RS, Sisomicin Sulfate RS, Sodium Alendronate Hydrate RS, Sodium Citrate Hydrate RS, Sodium Cromoglicate RS, Sodium Hyaluronate RS, Sodium Picosulfate Hydrate RS, Sodium Polystyrene Sulfonate RS, Sodium Salicylate RS, Sodium Valproate RS, Spectinomycin Hydrochloride RS, Spiramycin RS, Spironolactone RS, Stannous Fluoride RS, Stearic Acid RS, Streptomycin Sulfate RS, Strychnine Nitrate RS, Sulbactam RS, Sulbactam Sodium RS, Sulbenicillin Sodium RS, Sulfamethizole RS, Sulfamethoxazole RS, Sulfasalazine RS, Sulfinpyrazone RS, Sulpiride RS, Sultamicillin Tosylate RS, Suxamethonium Chloride Hydrate RS, Suxamethonium Chloride RS, Swertiamarin RS, Tamoxifen Citrate RS, Tamsulosin hydrochloride RS, Tansinone Ii A RS, Tegafur RS, Teicoplanin RS, Telmisartan RS, Temazepam RS, Tenoxicam RS, Terazosin Hydrochloride RS, Terbutaline Sulfate RS, Terconazole RS, Testosterone Enanthate RS, Testosterone Propionate RS, Tetracycline Hydrochloride RS, Tetrahydropalmatine RS, 2,3,5,4'-Tetrahydroxystilben-2-O-β-D-Glucoside RS, Tetrahydrozoline Hydrochloride RS, Theophylline RS, Thiamazole RS, Thiamine Hydrochloride RS, Thiamphenicol RS, Thiamylal RS,

Thiamylal Sodium RS, Thianthol RS, Thiopental Sodium RS, Thioridazine Hydrochloride RS, L-Threonine RS, Thrombin RS, Thymol RS, Tiapride Hydrochloride RS, Tiapride N-Oxide RS, Tiaprofenic Acid RS, Ticarcillin Sodium RS, Ticlopidine Hydrochloride RS, Timolol Maleate RS, Tinidazole RS, Tipepidine Hibenzate RS, Tipepidine Hibenzate RS, Tobramycin RS, Tocopherol Acetate RS, Tocopherol Calcium Succinate RS, Tocopherol RS, Tocopherol Succinate RS, Tofisopam RS, Tolazoline Hydrochloride RS, Tolbutamide RS, Tolfenamic Acid RS, Tolnaftate RS, Torsemide RS, Tramadol Hydrochloride RS, Tranexamic Acid RS, Trapidil RS, Triamcinolone Acetonide RS, Triamcinolone RS, Triamterene RS, Trichlormethiazide RS, Triclosan RS, Triflusal RS, Trihexyphenidyl Hydrochloride RS, Trimebutine Maleate RS, Trimetazidine Hydrochloride RS, Trimetoquinol Hydrochloride Hydrate RS, L-Tryptophan RS, Tubocurarine Chloride Hydrochloride Hydrate RS, Tulobuterol Hydrochloride RS, Ubidecarenone RS, Uracylarabinoside RS, Ursolic Acid RS, Vancomycin Hydrochloride RS, Varsartan RS, Verapamil Hydrochloride RS, Vigabatrin RS, Vinblastine Sulfate RS, Vincristine Sulfate RS, Vinorelbine Tartrate RS, Voglibose RS, Woogonin RS, Xanthanoic Acid RS, Xanthone RS, Xylitol RS, Zaltoprofen RS, Zolpidem Tartrate RS

The Korean Pharmacopoeia Reference Medicinal Plants Materials are as follows:

Achyranthes Root RMPM, Acori Graminei Rhizoma RMPM, Adenophorae Radix RMPM, Agastachis Herba RMPM, Akebia Stem RMPM, Alisma Rhizome RMPM, Anemarrhena Rhizome RMPM, Anethi Fructus RMPM, Angelica Dahurica Root RMPM, Angelica Gigas Root RMPM, Aomum Fruit RMPM, Apricot Kernel RMPM, Aralia Continentalis Root RMPM, Areca Seed 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, Cassia Bark RMPM, Chelidonii Herba 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

Captopril disulfide RS, carvedilol related substance I RS {(2RS-1-benzyl[2-(2-methoxyphenoxy)ethyl]amino-3-(9H-carbazol-4-yloxy)propan-2-ol)}, carvedilol related substance II RS {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 RS {1,1'-[[2-(2-methoxyphenoxy)ethyl]nitrile]bis[3-(9H-carbazole-4-yloxy)propane-2-ol]}, Cefaclor delta-3 isomer RS, ceftazidime high moleuclar polymer RS, cetirizine related substance I RS {(RS)-1-[(4-chlorophenyl)phenylmethyl] piperazine}, chenodeoxycholic acid RS, 4-Chlorobenzenesulfoneamide RS, 2-Chlorobenzoic acid RS, 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one-4-oxide RS, 3-Chloro-2-methylaniline RS, 2-Chloro-4-N-furfurylamino-5-sulfamoylbenzoic acid RS, (2-chlorophenyl)-diphenylmethanol RS, cilazapril related substance I RS {1,1-dimethylethyl(1S,9S)-9-[[(S)-1-(ethoxycarbonyl)-3-phenypropyl]amino]-10-oxoocatahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-carboxylate}, cilazapril related substance II RS {(1S,9S)-9-[[(S) -1-carboxy-3-phenylpropyl]amino]-10-oxooctahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid}, cilazapril related substance III RS {ethyl(1S,9S)-9-[[(S) -1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6H-pyridazino[1,2-a][1,2]diazepine-1-Carboxylate}, cilazapril related substance IV RS {(1S,9S)-9-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-10-oxooctahydro-6H-pyridizano[1,2-a][1,2]diazepine-1-carboxylic acid}, cinchonidine RS, cinchonine RS, ciprofloxacin Ethylenediamine derivative RS, cis-4-aminomethylcyclohexane-1-Carboxylic acid RS, 1-cla-1H-tetrazol-5-thiol RS, clenbuterol related substances I RS {1-(4-amino-3,5-dichlorophenyl)-2-[(1,1-dimethylethyl) amino]ethanol (cienbuterol-ketone)}, clomiphene related substance I RS {(E,Z)-2-[4-(1,2-diphenylethenyl)phenoxy]-N,N-deethyl ethaneaminehydrochloride}, clopidogrel related sub-

stance III RS {methyl(-)-(R)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine -5(4H)-acetate, hydrogen sulfate}, clopidogrel related substance I RS {(+)-(S)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine -5(4H)-acetic acid}, clopidogrel related substance II RS {methyl(±)-(o-Chlorophenyl)4,5-dihydrothieno[2,3-c]pyridine -6(7H)-acetate,hydrochloride}, Cyclobenzaprine hydrochloride RS, dermatan sulfate RS, Dibenzosuberone RS {10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-one}, Diclofenac Sodium RS, diethylene glycol RS, 3,4-dihydro-6-hydrocy-2(1H)-quinoline RS, diosmine related substance VI RS {5,7-dehydrocy-2 -(3-hydrocy-4-methoxyphenyl)-4H-l-benzopyran-4-one (diosmetin)}, diosmine related substance I RS {1-(3-hydroxy-4-methoxyphenylethanone(acetoisovanillone)}, diosmine related substance II RS {(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 RS {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 RS {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 RS {7-[[6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-Glaucopyranosyl]oxy]-5-hydrocy-2-(4-methoxyphenyl)-4H-l-benzopyran-4-one (linarin)}, 1,1-diphenyl-4-piperidino-1-butene hydrochloride RS, ebastine related substance I RS {diphenylethanol(benzhydrol)}, ebastine related substance II RS {1-[4-(1,1-dimethylethyl)phenyl]ethanol}, ebastine Related Substance III RS [4-(Diphenylmethoxy)piperidine], ebastine Related Substance IV RS {1-[4-(1,1-Dimethylethyl)phenyl]-4-(4-hydroxypiperidine-1-yl)butan-1-one], ebastine related substance V RS {1-[4-(1,1-demethylpropyl)phenyl]-4-[4-(diphenylmethoxy)piperidine-1-yl]butane-1-one}, ebastine related substance VI RS {1-[4-(1,1-demethylethyl)phenyl]-4-[cis-4-(diphenylmethoxy)-1-oxydopiperidine-1-yl]butane-1-one}, ebastine related substance VII RS {1-[4-(1,1-demethylethyl)phenyl]-4-[trans-4-(diphenylmethoxy)-1-oxydopiperidine-1-yl]butane-1-one}, econazole nitrate RS, enalaprilate RS, 4-epianhydrotetracyclinehydrochloride RS, epilactose RS, epitiostanol RS, erythromycin A iminoether RS, 2-ethyl-2-phenylmalonamide RS, etodolac related substance I RS {etodolac dimer}, fenofibrate related substance I RS [(4-chlorophenyl)(4-hydroxyphenyl) methanone], fenofibrate related substance I RS {(4-Chlorophenyl)(4-hydrocyphenyl)methanones}, fenofibrate related substance II RS {2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid (fenofibric acid)}, fenofibrate related substance III RS {1-methylethyl 2-[[2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoyl]oxy]2-methylpropanoate}, fenofibrate related substance IV RS {(3RS)-3-[4-(4-Chlorobenzoyl)phenoxy]butane-2-one}, fenofibrate related substance V RS {methyl 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoate}, fenofibrate related substance VI RS {ethyl 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoate}, fenofibrate related substance VII RS{(4-Chlorophenyl)[4-(1-methylethoxy)phenyl]methanones}, fenticonazole related substance I RS {(RS)-1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-3-[4-(phenylsulphanyl)benzyl]imidazolium nitrate}, flumequine RS and flumequine related substance I RS {(RS)-9-fluoro-5-methyl-1-oxo-6,7-dihydro-1H,5H-benzo[i.j]quinolizine-2-carboxylate (flumequine ethyl ester)], 9α-fluorine-11β-hydrocy-16β-methyl-3-oxo-androsta-1,4-diene-17(R)-Spiro-2'-[4'-Chloro-5'-ethylfuran-3-(2'H)-one] RS, 4[4-(fluorine-11β-hydrocy-16β-methyl-3-oxo-androsta-1,4-diene-17-Spiro-2'-(9α-)hydrophenylmethyl)-1-Piperidinyl]-1-butanone RS, fluoxetine related substances I RS {N-methyl-3-pheny-[(a,a,a-(trifluoro-me-tolyl)oxy)propylamine hydrochloride}, fluoxetine related substances II RS {N-methyl-3-phenylpropylamine}, fluticasone propionate related substance I RS {[6α,9α-difluoro-11B-hydroxy-16α-methyl-3-oxo-17α-propanoyloxyandrosta-1,4-diene-17B-carbonyl sulfenicc acid}, fluticasone propionate related substance II RS {6α,9α-difluorine-11B-hydrocy-16α-methyl-2',3,4'-trioxo-17α-Spiro(androsta-1,4-diene-17,5'-(1,3)oxathiolane)}, fluticasone propionate related substance III RS {S-fluorinemethyl 17α-acetyloxy-6α,9α-difluorine-11B-hydrocy-16α-methyl-3-oxo-androsta-1,4-diene-17B-carbothioate}, fluticasone propionate related substance IV RS {S-methyl 6α,9α-difluoro-11B-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-diene-17B-carbothioate}, fluticasone propionate related substance V RS {6α,9α-difluorine-11B,17α-dihydrocy-16α-methyl-3-oxo-androsta-1,4-diene-17B-carboxylic acid 6α,9α-difluoro-17B-(fluoromethylthio)carbonyl-11B-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17α-yl ester}, formoterol fumarate dihydrate RS, galactitol RS [dulctol], galactose RS, gentamycin B RS, gitoxin RS, gliclazide related substance I RS [2-nitroso-octahydrocyclopenta[c]pyrrole], gliclazide related substance II RS [1-(hexahydrocyclopenta[c] pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea], guanine RS, hydrochlorothiazide related substance I RS {4-amino-6-Chloro-1,3-benzenedesulfoneamide}, 1-(2-hydrocyethyl)-1H-tetrazol-5-thiol RS, 5-hydrocymethyl-2-furaldehyde RS, hyoscine Hydrobromide RS, Imipramine Hydrochloride RS, iodixanol related substance I RS {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 RS {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-

dehydrocypropyl)-2,4,6-triiodo-1,3-benzenedecarboxamide}, iodixanol related substance IV RS {2-[[acetyl[3,5-bis[[2,3-dehydrocypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dehydrocypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-decarboxamide}, iodixanol related substance V RS {4-acetyl-2-[[acetyl[3,5-bis[[2,3-dehydrocypropyl)amino]carbonyl]-2,4,6-triiodophenyl]amino]methyl]-N,N'-bis(2,3-dehydrocypropyl)-2,3-dihydro-5,7-diiodo-4H-1,4-benzoxazine-6,8-decarboxamide}, iodixanol related substance VI 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 VII RS, iohexol related substance I RS {5-(amino)-N,N,-bis(2,3-dihydroxypropyl)-2,4,6-triiod-1,3-benzenedicarboxy-amide)}, iohexol related substance II RS {5-(acetylamino)-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide}, iohexol related substance III RS {N,N'-bis(2,3-dihydroxypropyl)-5-nitro-1,3-benzenedicarboxamide}, iohexol related substance IV RS, iopromide related substance I RS {[5-(acetylamino)-N,N'-bis(2,3-dihydroxypropyl-2,4,6-triiodo-N-methyl-1.3-benzene-dicarboxamide}, iopromide related substance II RS, isobutylpiperidone RS, isonicotinic acid amide RS, isopromethazine hydrochloride RS, isradipine related substance I RS {isopropylmethyl 4-(4-benzofurzanyl)-2,6-dimethyl-3,5-pyridinedicarboxy-late}, Lacidipine related substance I RS {diethyl(E)-4-{2-[2-tert-butoxycarbonyl)viny]phenyl}-2,6-dimethylpyridine -3,5-diCarboxylate}, lactose anhydride RS, lamivudine resolution mixture I RS, lamivudine resolution mixture II RS, lansoprazole related substance I RS {2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulfonyl benzimidazole}, L-arabinitol {l-arabitol 1,2,3,4,5-pentanephenol} RS, letrozole related substance I RS {4,4'-(1H-1,3,4-triazol-1-ylmethylene)dibenzonitrile}, levodropropizine related substance II RS {(2RS)-oxiran-2-yl]methanol (glycidol)}, levodropropizine related substance II RS {1-phenylpiperazine}, levodropropizine related substance III RS {(2R)-3-(4-phenylpiperazin-1-yl)propane-1,2-diol (dextrodropropizine)}, lithocholic acid RS, lovastatin related substance I RS {dihydrolovastatin}, maleic acid RS, medroxyprogesterone acetate related substance I RS, meloxicam related substance I RS {ethyl-4-hydrocy-2-methyl-2H-1,2-benzothiazine-3-Carboxylate-1,1-deoxide}, meloxicam related substance II RS {5-methylthiazole-2-ylamine}, meloxicam related substance III RS {4-hydrocy-2-methyl-N-ethyl-N'-(5-methyl-1,3-thiazole-2-yl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide}, meprobamate RS, mesalazine related substance I RS {2-aminophenol), mesalazine related substance II RS {4-aminophenol), mesalazine related substance III RS {3-aminophenol), mesalazine related substance IV RS {aniline), mesalazine related substance V RS {3-aminobezoic acid), mesalazine related substance VI RS {2,5-dihydroxybezoic acid), mesalazine related substance VII RS {salicylic acid), methotrexate related substance III RS {4-{[(2,4-deaminopteridine-6-yl)methyl]methylamino}benzoic acid 1/2hydrochloride}, methotrexate related substance I RS {(S)-2-{4-[(2,4-deaminopteridine-6-yl)methylamino]benzamido}pentanedioate}, methotrexate related substance II RS {(S)-2-(4-{[(2-amino-4-oxo-1,4-dehydropteridine-6-yl)methyl](methyl)amino}benzamido)pentanedioate}, 1-methylazepan-4-one hydrochloride RS, 1-methyl-4-(2-benzoylhydrazino)azepan hydrochloride RS, 3-O-methylmethyldopa RS, 2-methyl-5-nitroimidazole RS, methylphenidate Hydrochloride erythro isomer RS, N-(2-hydrocyl ethyl)isonicotinamideamide nitric ester RS, nabumetone related substance I RS [1-(6-methoxy-2-naphthyl)-but-1-ene-3-one], N-aminohexamethyleneimine RS, nifedipine nitrophenylpyridine analog RS {dimethyl4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-diCarboxylate}, nifedipine nitrosophenylpyridine analog RS {dimethyl4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-diCarboxylate}, nifuroxazide related substance I RS {4-hydroxybenzohydrazide}, nifuroxazide related substance II RS {methyl p-hydroxybenzoate}, nifuroxazide related substance III RS {(5-nitrofuran-2-yl)methylenediacetate}, nifuroxazide related substance IV RS {1,2-bis[(5-nitrofuran-2-yl)methyllen]diazan(5-nitrofurfuralazine)}, Nimodipine related substance I RS {2-methoxyethyl-methylethyl-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate}, nitrilotriacetic acid RS, N-Methylpyrrolidine RS, ondansetron related substance I RS (1,2,3,9-Tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one) RS, ondansetron related substance II RS(1,2,3,9-Tetrahydro-9-methyl-4H-carbazol-4-one) RS , ondansetron related substance III RS {3-[(Dimethylamino)methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one} RS, ondansetron related substance IV RS {6,6'-Methylene-bis-[(1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)-methyl]-4H-carbazol-4-one} RS, orphenadrine Related Substance I RS {(RS)-N,N-Dimethyl-2-[(3-methylphenyl) phenylmethoxy]ethanamine} RS, oversulfated chondroitin sulfate RS, oxaliplatin related substance I RS (oxalic acid), oxaliplatin related substance II RS [(SP-4-2-)-diaqua[(1R,2R)-cyclohexane-1,2-diamine-κN,κN']platinum(diaquodiaminocyclohexane platinum)], oxaliplatin related substance III RS {(OC-6-33)-[(1R,2R)-cyclohexane-1,2-diamine-κN,κN'][ethanedioato(2-)-κO1,κO2]dihydroxyplatinum} RS, oxaliplatin related substance IV RS {(SP-4-2)-[(1S,2S)-Cyclohexane-1,2-diamine-κN,κN'][ethanediato(2-)-κO1,κO2]platinum} RS, oxaliplatin related substance V RS {(SP-4 -2)-de-μ-oxobis[(1R,2R)-cyclohexane-1,2-diamine-

κN,κN']deplatinum}, paclitaxel related substance II RS {10-deacetyl-7-epipaclitaxel}, paclitaxel related substances I RS {10-deacetyl-7-epipaclitaxel}, paclitaxel related substances I RS{cephalomannine}, p-aminobenzoylglutamic acid RS, p-aminobenzoylglutamine RS, paroxetine related substance I RS {(3S,4R)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl)piperidine((+)-trans-paroxetine)}, paroxetine related substance II RS {(3S,4R)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-ethoxyphenyl)piperidine}, paroxetine related substance III RS {(3S,4R)-3- [(1,3-benzodioxol-5-yloxy)methyl]-4-phenylpiperidine (desfluoroparoxetine)}, α-phenyl-2-piperidineacetic acidhydrochloride RS, phenytoin related substance I RS {Diphenyl glycine}, phenytoin related substance II RS {Diphenyl hydantoic acid}, phloroglucinol related substance I RS {pyrogallol}, phloroglucinol related substance II RS {phloroglucide}, piperidine hydrochloride RS, piracetam related substance I RS {2-pyrrolidone}, primidone related substance I RS [2-ethyl-2-phenylpropanediamide], primidone related substance II RS {2-phenylbutyramide}, propofol related substance III RS {3,3',5,5'-tetrakis(1-methylethyl)biphenyl-4,4'-diol}, propofol related substance I RS {2,6-bis(1-methylethyl)-1,4-benzoquinone}, propofolrelated substanceIIRS{2-(1-methylethoxy)-1,3-bis(1-methylethyl)benzene} , ramipril related substance I RS {[2S,3aS,6aS)-1-[(S)2-[[(S)1-(methoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid}, ramipril related substance II RS {[(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 RS {[(2S,3aS,6aS)-1-[(S)2-[[(S)1-(ethoxycarbonyl)-3-cyclohexyl propyl]amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid}, ramipril related substance IV RS {[ethyl(2S)2-(3S,5aS,8aS,9aS)-3-methyl-1,4-deoxodecahydro-1H-cyclopenta[e]pyrrolo[1,2-a]pyrazin-2-yl]-4-phenylbutanoate}, ranitidine related compound I RS, ranitidine related compound II RS, ranitidine related compound III RS, repaglinide related substance I RS {(S)-3-methyl-1-[2-(1-piperidinyl)phenyl]butylamine,N-acetyl-L-glutamate}, repaglinide related substance II RS {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 RS, Sorbitol RS, telmisartan related substance II RS {4'-[(1,7'-demethyl-2'propyll-1H,1'H-2,5'-nonbenzo[d]imidazole-1'-yl)methyl]phenyl-2-Carboxylic acid}, Terazosin related substance I RS {1-(4-amino-6,7-demethoxy-2-quinazolinyl)piperazine,dehydrochloride}, Terazosin related substance II RS {1-(4-hydrooxy-6,7-demethoxy-2-quinazolinyl)-4-[(tetrahydro-2furanyl) carbonyl]piperazine }, Terazosin related substance III RS {1,4-bis(4-amino-6,7-demethoxy-2-quinazolinyl)piperazine ,dehydrochloride}, terbutaline related substance I RS {3,5-dehydrocy-ω-t-butylaminoacetophenonesulfate}, tetrahydro-2-furan carboxylic acid RS, 1-[(tetrahydro-2-furanyl)piperazine RS, tiapride related substance I RS {N,N-deethyl ethane-1,2-diamine}, tiaprofenic acid related substance I RS [(2RS)-2-(5-benzoylthiopene-3-yl)propanosan], tiaprofenic acid related substance II RS {(5-ethylthiophene-2-yl)phenylmethanones}, tiaprofenic acid related substance III RS {1-(5-benzoylthiophene-2-yl)ethanone}, tolfenamic acid related substance I RS {2-chlorobenzoic acid}, tolfenamic acid related substance II RS {2-chloro-2-methylaniline}, torsemide related substance I RS {4-[(3-methylphenyl)amino]-3-pyridinesulfonamide}, torsemide related substance II RS {N-[(n-butylamino)carbonyl]-4-[(3-methylphenyl)amino]-3-pyridinesulfoneamide}, torsemide related substance III RS {N-[ethylamino)carbonyl]-4-[(3-methylphenyl) amino]-3-pyridinesulfoneamide}, tramadol hydrochloride related substance I RS {(2RS)-2-[(dimethylamino)methyl]cyclohexanone}, tramadol hydrochloride related substance II RS {(1RS,2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol}, tretinoin RS, tribenoside related substance I RS {3,5,6-tri-O-benzyl-1,2-O-(1-methyl-ethylidene)-α-d-glucofuranose}, tribenoside related substance II RS {benzaldehyde}; tribenoside related substance III {dibenzylether}, tribenoside related substance IV RS {3,5-de-O-benzyl-1,2-O-(1-methylethylidene)-α-d-Glaucofuranose}, triflusal related substance I RS (2-acetoxyterephthalic acid), triflusal related substance II RS [4-(trifluoromethylsalicylic acid)], Trihexyphenidyl related substance I RS {1-phenyl-3-(piperidi-1-yl)propan-1-one}, urea RS, valsartan related substance I RS {R-N-valery-N-([2'-(1H-tetrazol-5-yl)bifen-4-yl]methyl)valine}, valsartan related substance II RS {S-N-Butyryl-N-(2'-(1H-tetrazol-5-yl)bifen-4-yl]methyl)-valine}. valsartan related substance III{S-N-valery-N-([2'-(1H-tetrazol-5-yl)bifen-4-yl]methyl)-valine benzyl ester}, vinorelbin related substance I RS {4-O-deacetylvinorelbin}, 5-vinyl-2-pyrrolidone RS, xanthantanoic acid RS, xanthon RS, zolpidem related substance I RS {N,N-Dimethyl-2-[7-methyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-3-yl]acetamide} RS

(2) Reagents and Test Solutions

Absorbent cotton [Same as the namesake monograph in Part II].

Acenaphthene C_2H_{10}, White to pale yellowish white crystals or crystalline powder, having a characteristic aroma. Freely soluble in ether or chloroform, soluble in a cetonitrile, sparingly soluble in methanol, and practically insoluble in water.

Identification Determine the infrared absorption spectrum according to the paste method under the Infrared Spectrophotometry, with 5 mg of acenaphthene:

it exhibits the absorptions at the wave numbers at about 1605 cm^{-1}, 840 cm^{-1}, 785 cm^{-1} and 750 cm^{-1}

Melting point 93 ~ 96 °C.

Purity Dissolve 0.1 g of Acenaphthene in 5mL of chloroform, and use this solution as the test solution. Perform the test with 2 μL of the test solution as directed under the Gas Chromatography under the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of Acenaphthene by the area percentage method: it shows a purity of not less than 98.0 %.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column, about 3 mm in inside diameter and about 2 m in length, packed with 10 % of polyethylene glycol 20 M supported on 150-μm to 180-μm siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 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, which is prepared by adding chloroform to 1.0 mL of the test solution to make 100 mL, composes 5 % to 15 % of the full scale.

Time span of measurement: About 3 times as long as the retention time of acenaphthene after the solvent peak.

Acetal $C_6H_{14}O_2$ A clear and colorless volatile liquid. Miscible with water and with ethanol (95).

Refractive index n_D^{20} about 1.382

Boiling point about 103 °C

Specific gravity d_{20}^{20} about 0.824

Acetaldehyde CH_3CHO [First class].

Acetanilide $C_8H_9NO_2$ White, crystals or crystalline powder.

Melting Point: 114-117

***p*-Acetanisidede** $C_9H_{11}NO_2$ 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 ~ 132

Content not less than 98.0 %.

Assay Dissolve 0.1 g of *p*-acetanisidide in 5 ml of ethanol (95). Perform the test with 2 μL of this solution as directed under the Gas Chromatography according to the following conditions, and determined the area of each peak by the automatic integration method.

$$\text{Content} = \frac{\text{peak area of } p\text{-acetanisidide}}{\text{total of all peak areas}} \times 100$$

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass tube 3 mm in inside diameter and 2 m in length, packed with acid treated and silanized siliceous earth for gas chromatography coated with alkylene glycol phthalate ester for gas chromatography in 1 % (177 - 250 μm in particle diameter)

Column temperature: A constant temperature of about 210 °C

Carrier gas: Nitrogen

Flow rate: Adjust to a constant flow rate of between 30 and 50mL per mite and so that the retention time of p-acetanisidide is between 11 and 14 minutes,

Time span of measurement: About 3 times as long as the retention time of p-acetanisidide after the solvent peak.

Acetate buffer solution, pH 4.5 Dissolve 63 g of anhydrous sodium acetate in a suitable amount of water, add 90 mL of acetic acid (100) and water to make 1000 mL

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. (100) (3 in 2500).

Acetic acid See acetic acid (31).

Acetic acid-ammonium acetate buffer solution, pH 3.0 Add acetic acid (31) to ammonium acetate TS, and adjust the pH to 3.0.

Acetic acid-ammonium acetate buffer solution, pH 4.5 Dissolve 77 g of ammonium acetate in about 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000mL.

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, dilute Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

Acetic acid for nonaqueous titration Acetic acid (100), meeting with following requirement.

Purity Acetic anhydride -Dissolve 1.0 g of aniline in acetic acid for nonaqueous titration to make 100 mL, and use this solution as the test solution. Pipet 25 mL of the test solution, titrate with 0.1 mol/L perchloric acid VS, and designate the consumed volume as A (mL). A is not less than 26 mL. Pipet 25 mL of the test solution, add 75 mL of glacial acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL) (potentiometric titration). A (mL) - B (mL) is not more than 0.1 (mL) (not more than 0.001 g/dL).

Acetic acid-potassium acetate buffer solution, pH 4.3 Dissolve 14 g of potassium acetate in 20.5 mL of acetic acid (100), and add water to make 1000 mL.

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) drop-wise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, 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, pH 4.5, for iron limit test Dissolve 75.4 mL of acetic acid (100) and 111 g of sodium acetate trihydrate in 1000 mL of water.

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) drop-wise, 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 5.5 Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) drop-wise, and add water to make 100 mL.

Acetic acid-sodium acetate burr solution, pH 5.6 Dissolve 12 g of sodium acetate trihydrate in 0.66 mL of acetic acid (100) and water to make 100 mL.

Acetic acid-sodium acetate buffer solution, 1 mol/L, pH 5.0 To sodium acetate TS, add dilute acetic acid, and adjust the pH to 5.0.

Acetic acid-sodium acetate TS Mix 17mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

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, (100) CH_3COOH, [Special class].

Acetic acid (100)-sulfuric acid TS To 5mL 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 anhydride $(CH_3CO)_2O$ [Specialclass].

Acetic anhydride-pyridine TS Place 25 g of acetic anhydride in a 100 mL volumetric flask, add pyridine to make 100 mL, and mix well. Preserve in light-resistant containers, protected from air. This solution may be used even if it becomes colored during storage.

Acetone CH_3COCH_3, [Specialclass].

Acetone for nonaqueous titration Add potassium permanganate to acetone in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distil by using a fractionating column under protection from moisture, and collect the fraction distilling at 56 °C.

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, and add 3mL of acetic acid (100), 2 mL of acetylacetone, and water to make 1000 mL. Prepare before use.

Acetylene See dissolved acetylene.

Acidic ferric chloride TS See iron (III) 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.

Acid-treated gelatin See gelatin, acid-treated.

Acrinol $C_{15}H_{15}N_3O \cdot C_3H_6O_3H_2O$ [Same as the namesake monograph].

Acrylamide $CH_2CHCONH_2$ Pale yellow crystalline powder.
Melting point 83 - 86 °C
Content not less than 97.0 %

Activated alumina Aluminum oxide with specially strong adsorptive activity.

Activated charcoal [Same as the monograph in Part II, Medicinal Carbon].

Adipic acid $C_4H_8(COOH)_2$, White crystals or crystalline powder. Freely soluble in ethanol (95), and sparingly soluble in water.

Melting point between 151 °C and 154 °C
Content not less than 98.0 %. Assay Weigh accurately about 1 g of adipic acid, and 100mL of water, dissolve by warming, cool, and titrate 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. Same as the monograph in Part II, Agar or Agar Powder. Loss on drying is not more than 15 %].

Agar medium, ordinary See ordinary agar medium.

Agar slant Dispense portions of about 10mL of ordinary agar medium into test tubes, and sterilize by autoclaving. 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.

L-Alanine $C_3H_7NO_2$ [Special class]

Albumin TS Carefully separate the white from the yolk of a fresh hen's egg. Shake the white with 100mL of water until the mixture is thoroughly mixed, and filter. Prepare before use.

Alcoholic Hydroxylamine TS See Hydroxylamine TS, alcoholic

Aldehyde dehydrogenase Each mg contains not less than 2 enzyme activity units. White powder.
Assay Dissolve about 0.02 g of aldehyde dehydrogenase, accurately weighed, in 1 mL of water, add ice-cold solution of bovine serum albumin (1 in 100) to make exactly 200 mL, and use this solution as the test solution. Place in a spectrophotometric cell 2.50mL of pyrophosphate buffer solution, pH 9.0, 0.20 mL of a solution prepared by dissolving 0.0200 g of β-nicotinamide adenine dinucleotide (NAD) to make exactly 1 mL, 0.10 mL of a pyrazole solution (17 in 2500) and 0.10mL of the test solution, stir, stopper tightly, and allow to stand at 25 ± 1 °C for 2 minutes. To this solution, add 0.01mL of an acetaldehyde solution (3 in 1000), stir, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under the Ultraviolet-visible Spectrophotometry, and calculat e 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 conducted under the conditions of the Procedure.

$$\text{Enzyme activity unit (unit/mg) of aldehyde dehydrogenase} = \frac{2.91 \times \Delta A \times 200}{6.3 \times W \times 0.1 \times 1000}$$

W: Amount of sample taken (g)

Aldehyde dehydrogenase TS Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10mL of water. Prepare before use.

Aldehyde-free ethanol See ethanol, aldehyde-free.

Alizarin complexone $C_{19}H_{15}NO_8$ (1,2-Dihydroxyanthraquino-3-ylmethylamine-N,N-diacetate), A yellow-brown powder. Soluble in ammonia TS, and practically insoluble in water, ethanol (95) or ether.
Sensitivity Dissolve 0.1 g of alizarin complexone by adding 2 drops of ammonia solution (28), 2 drops of ammonium acetate TS, and 20 mL of water. To 10mL of this solution, add acetic acid-potassium acetate burr solution, pH 4.3. to make 100mL. 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 hexahydrate TS, stir, and observe under scattered light after 1 minute: a blue-purple color is produced, and the color of the control solution is red-purple. Use a solution prepared in the same manner, to which 1 drop of water is added in place of a solution of sodium fluoride, as the control solution.

Alizarin complexone TS Dissolve 0.390 g of alizarin complexone in 20 mL of a freshly prepared solution of sodium hydroxide (I in 50), then add 800 mL of water and 0.2 g of sodium acetate, and dissolve. Adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid VS, and add water to make l000 mL.

Alizaria 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 100mL, and filter if necessary.

Alizarin S See alizarin red S.

Alizarin S TS See alizarin red S TS.

Alizarin yellow GG $C_{13}H_8N_3NaO_5$ [Special class].

Alizarin yellow GG-thymolphthalein TS Mix 10mL of alizarin GG TS with 20mL of thymolphthalein TS.

Alizarin yellow GG TS Dissolve 0.1 g of alizarin yellow GG in 100mL of ethanol (95), and filter, if necessary.

Alkaline blue tetrazolium TS See blue tetrazolium TS, alkaline.

Alkaline copper TS See copper TS, Alkaline

Alkaline cupric sulfate solution See copper (II) sulfate solution, alkaline.

Alkaline 1,3-Dinitrobenzene TS See 1,3-Dinitrobenzene TS, alkaline

Alkaline hydroxylamine TS See hydroxylamine TS, alkaline.

Alkaline m-dinitrobenzene TS See 1,3-dinitrobenzene TS, alkaline.

Alkaline glycerin TS To 200 g of glycerin add water to make 235 g and add 142.5 mL of sodium hydroxide TS and 47.5 mL of water.

Alkaline picric acid TS See 2,4,6-trinitrophenol TS, alkaline.

Alkaline Phosphatase See Phosphatase, Alkaline

Alkaline Phosphatase TS See Phosphatase TS, Alkaline

Alkaline potassium ferricyanide TS See potassium hexacyanoferrate (III) TS, alkaline.

Alkaline 2,4,6-Trinitropbenol TS See 2,4,6-Trinitropbenol TS, alkaline

Aluminon $C_{22}H_{23}N_3O_9$ [Special class].

Aluminon TS Dissolve 0.1 g of aluminon in water to make 100 mL, and allow this solution to stand for 24 hours.

Aluminum Al [Special class].

Aluminum chloride See aluminum (III) chloride hexahydrate.

Aluminum (III) chloride bexahydrate $AIC_{13}.6H_2O$ [Special class].

Aluminum potassium sulfate 12-hydrate $A1K(SO_4)_2 \cdot 12H_2O$ [Special class].

Aluminum oxide Al_2O_3, White crystals, crystalline powder, or powder. Boiling point: About 3000 °C. Melting point: About 2000 °C.

Amidosulfuric acid (Standard reagent) $HOSO_2NH_2$[Standard substance for volumetric analysis].

Aminoacetic acid See glycine.

***p*-Aminoacetophenone** See 4-aminoacetophenone.

***p*-Aminoacetophenone TS** See 4-aminoacetophenone TS.

4-Aminoacetophenone $H_2NC_6H_4COCH_3$ [p-Aminoacetophenone, Special class]. Lightyellow, crystals or crystalline powder, having a characteristic odor.
Melting point 105 ~ 108 °C

4-Aminoacetophenone TS Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100mL.

4-Aminoantipyrine $C_{11}H_{13}N_3O$ [Special class].

4-Aminoantipyrine hydrochloride $C_{11}H_{13}N_3O.HCl$, A light yellow crystalline powder. It dissolves in water. Melting point: Between 232 °C and 238 °C (decomposition).
Purity Clarity of solution - Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water: the solution is almost clear.
Content between 100.6 % and 108.5 %. Assay Weigh accurately about 0.5 g of 4-amino-antipyrine hydrochloride, dissolve in 50 mL of water, and if necessary, neutralize with 0.1 mol/ L sodium hydroxide VS (indicator: red litmus paper). Add 4 drops of dichlorofluorescein TS, and titrate with 0.1 mol/L silver nitrate VS.

EachmL of 0.1 mol/L silver nitrate VS
= 23.970 mg of $C_{11}H_{13}N_3O.HCl$

4-Aminoantipyrine hydrochloride TS Dissolve 1 g of 4-aminoantipyrine hydrochloride in water to make 50mL.

4-Aminoantipyrine TS Dissolve 0.1 g of 4-aminoantipyrine in 30 mL of water, add 10 mL of a solution of sodium carbonate decahydrate (1 in 5), 2 mL of sodium hydroxide TS and water to make 100 mL. Prepare before use.

Aminobenzoate derivatization TS To 0.28 g of ethyl aminobenzoate add 600 μL of methanol, warm at about 50 °C to dissolve, and add 170 μL of acetic acid and 145 μL of borane-pyridine complex.

***p*-Aminobenzoic acid** See 4-aminobenzoic acid.

4-Aminobenzoic acid $C_7H_7NO_2$, A white to very pale yellow crystalline powder. A solution of p-aminobenzoic acid in ethanol (95) (1 in 100) is clear.

4-Amino-6-chloro-1,3-benzenedisulfonamide $C_6H_8ClN_3O_4S_2$, White powder. Insoluble in water or chloroform and soluble in ammonia TS. Determine the absorption spectrum of a solution of 4-Amino-6-chloro-1,3-benzenedisulfonamide in methanol (1 in 200000) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits maximum at 223 nm and at 265 nm.
Absorbance E (265 nm) : about 64.0.
Residue on ignition not more than 0.1 % (2 g).

2-Amino-5-chlorobenzophenone for thin-layer chromatography $C_{13}H_{10}ClNO$, Yellow ,crystalline powder.
Melting point between 97 °C and 101 °C
Purity Related substances Dissolve 0.010 g 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 spot other than the principal spot at the Rf value about 0.7 does not appear.

2-Aminoethanol $NH_2CH_2CH_2OH$[Special class].

***N*-Aminohexamethyleneimine** $(CH_2)_6NNH_2$, A clear colorless to pale yellow liquid.
Refraction index n_D^{20} between 1.482 and 1.487
Specific gravity d_{20}^{20} between 0.936 and 0.942

4-Amino-N,N-diethylaniline sulfate $H_2NC_6H_4N(C_2H_5)_2 \cdot H_2SO_4 \cdot H_2O$, White to slightly colored powder. It dissolves in water.
Melting point between 173 °C and 176 °C.
Residue on ignition not more than 0.10 % (1 g).

4-Amino-N,N-dietbylaniline sulfate TS Dissolve 0.2 g of 4-amino-N JV-diethylaniline in water to make 100 mL. Prepare before use, protected from light.

4-(Aminomethyl)benzoic acid $C_8H_9NO_2$ A white powder.
Purity Dissolve 10 mg of 4-(aminomethyl)benzoic acid in 100mL of water, and use this as the sample solution. Pipet 1mL of the sample solution, add water to make exactly 20mL, and use this solution as the standard solution. Perform the test with exactly 20mL each of the sample solution and standard solution as directed under Liquid Chromatography according to the operating conditions as directed in the Purity (5) under Tranexamic Acid, and determine each peak area by the automatic integration method: each area of the peak other than 4-(aminomethyl)benzoic acid obtained from the sample solution is not larger than the peak area of 4-(aminomethyl)benzoic acid from the standard solution.

***m*-Aminophenol** See 3-aminophenol.

3-Aminopbenol $H_2NC_6H_4OH$ [First class]. White, crystals or crystalline powder.
Melting point between 121 °C and 125 °C
Content not less than 97.0 %.
Assay Weigh accurately about 0.2 g, dissolve in 50mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid VS potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid VS
= 10.91 mg of $H_2NC_6H_4OH$

***p*-Aminophenol hydrochloride hydrochloride** See 4-aminophenol hydrochloride

4-Aminophenol hydrochloride $HOC_6H_4.NH_2C1$ White to pale colored crystals. Freely soluble in water and in ethanol (95). Melting point: About 306 °C (with decomposition).
Content not less than 99.0 %.
Assay Weigh accurately about 0.17 g of 4-aminophenol hydrochloride, dissolve in 50mL of acetic acid for nonaqueous titration and 5mL of mercury (II) acetate TS for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid-dioxane VS (indicator: 1 mL of a-naphtholbenzeine TS). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid-dioxane VS
= 14.559 mg of $HOC_6H_4NH_2.HCl$

Storage Preserve in tight, light-resistant containers.

2-Amino-1-butanol $CH_3CH_2H(NH_2)CH_2OH$, Clear, colorless to light yellow liquid. Miscible with water and dissolves in methanol.
Refraction index n_D^{20} between 1.450 and 1.455.
Specific gravity d_{20}^{20} between 0.944 and 0.950.
Purity Related substances -Dissolve 0.050 g of 2-amino-1-butanol in 10mL of methanol, measured exactly, and perform the test with 12 μL of this solution as directed in the purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the R_f value of about 0.3 does not appear.

2-Amino-2-hydroxymethyl-1,3-propanediol $C_4H_{11}NO_3$ [Special class].

2-Amino-2-hydroxymethyl-1,3-propanediol TS Dissolve 1.5 g of 2-Amino-2-hydroxymethyl-1,3-propanedio in water to make 100 mL. Pipet 40 mL of this solution, add Dimethylsulfoxide to make 200 mL. Use within 4 hour.

1-Amino-2-naphthol-4-sulfonic acid $C_{10}H_9NO_4S$ [Special class].

1-Amino-2-naphthol-4-sulfonic acid TS Mix thoroughly 5 g of anhydrous sodium sulfite, 94.3 g of sodium bisulfite and 0.7 g of 1-amino-2-naphthol-4-sulfonic acid. Before use, dissolve 1.5 g of this mixture in water to make 10 mL.

***p*-Aminophenol** Fine, yellowish, crystalline powder. Slightly soluble in water and in ethanol (95).
Melting point between 187 °C and 189 °C

Aminopropylsilanized silica gel for pretreatment Preprared for pretreatment

L-2-Aminosuberic acid $C_8H_{15}NO_4$ White, crystals or crystalline powder. Odorless.
Optical rotation : +19.1 ~ +20.19(after drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm).
Loss on Drying not more than 0.3 % (1 g, 105 ºC, 2 hours).
Assay Weigh accurately about 0.3 g of L-2-aminosuberic acid, previously dried, add exactly 6mL of formic acid to dissolve, then add exactly 50mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentio-metric titration). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid VS
= 18.92 mg of $C_8H_{15}NO_4$

Ammonia-ammonium acetate buffer solution, pH 8.0 Add ammonium TS dropwise to ammoimum acetate TS, and adjust the pH to 8.0.

Ammonia-ammonium acetate buffer solution, pH 8.5 Dissolve 50 g of ammonium acetate in 800 mL of water and 200 mL of ethanol (95), and add ammonia solution (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 solution (28), dilute with water to make 1000 mL, and add ammonia solution (28) drop-wise 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 solution (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 solution (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 copper TS To 0.5 g of cupric carbonate, add 10mL of water, triturate, and add 10mL of ammonia solution (28).

Ammonia-Cyanide TS Dissolve 2 g of potassium cyanide in 15 mL of ammonia solution (28), and dilute with water to 100 mL.

Ammonia-ethanol TS To 20 mL of ammonia solution (28), add 100 mL of dehydrated ethanol (99.5).

Ammoaia gas NH_3, Prepare by heating ammonia solution (28).

Ammonia solution (28) NH_4OH [Ammonia Water, Special class]
Specific gravity about 0.90
Density 0.908 g/mL
Content between 28 % and 30 %

Ammonia TS To 400 mL of ammonia solution (28), add water to make 1000 mL (10 %)

Ammonia water See ammonia TS.

0.5 mol/L Ammonia water To 32.5 mL of ammonia solution (28) add water to make 1000 mL.

1 mol/L Ammonia water To 65 mL of ammonia solution (28) add water to make 1000 mL.

13.5 mol/L Ammonia water To exactly 9 mL of water, add ammonia solution (28) to make exactly 50 mL.

Ammonia water, strong See ammonia solution (28).

Ammonium acetate CH_3COONH_4 [Special class].

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 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 amidosulfate $NH_4OSO_2NH_2$ [Special class].

Ammonium amidosulfate TS Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

Ammonium carbonate [Special class].

Ammonium carbonate TS Dissolve 20 g of ammonium carbonate in 20 mL of ammonia TS and water to make 100 mL.

Ammonium chloride NH_4Cl [Special class].

Ammonium chloride-ammonia TS To ammonia solution (28), add an equal volume of water, and saturate this solution with ammonium chloride.

Ammonium chloride buffer solution, pH 10 Dissolve 5.4 g of ammonium chloride in water, and add 21 mL of ammonia solution (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 citrate See diammonium hydrogen citrate.

Ammonium dihydrogen phosphate $NH_4H_2PO_4$ [Special class].

Ammonium formate $HCOONH_4$ Colorless crystals. Very soluble in water.
Melting point 116-119 °C

Ammonium formate TS Dissolve 3.25 g of ammonium formate in water to make 1000mL

Ammonium formate buffer solution, 0.05 mol/L, pH 4.0 Dissolve 3.5 g of ammonium formate in 750mL of water, adjust to pH 4.0 with formic acid, and add water to make 1000 mL.

Ammonium hydrogen carbonate NH_4HCO_3 White or semi-transparency, crystals crystalline powder or masses, having an ammonia odor.

Ammonium Bisulfate (AmmoniumHydrogenSulfate) NH_4HSO_4 White crystals. Freely soluble in water; practically insoluble in alcohol, in acetone, and in pyridine.
Content Not less than 98 %.
Assay Dissolve about 0.3 mg, accurately weighed, in 50mL of a mixture of water and alcohol (25:25). Titrate with 0.1 mol/L sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide
= 11.51 mg of NH_4HSO_4.

Ammonium iron (II) sulfate hexahydrate $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ [Special class].

Ammonium iron (III) citrate [Same as the monograph Ferric Ammonium Citrate of the Korean Standards of Food Additives].

Ammonium iron (III) sulfate TS Dissolve 8 g of ammonium iron (III) sulfate 12-hydrate in water to make 100 mL.

Ammonium iron(Ⅲ) sulfate TS, acidic Dissolve 20 g of ammonium iron (Ⅲ) sulfate 12-hydrate in a suitable amount of water, add 9.4 mL of sulfuric acid, and add water to make 100 mL.

Ammonium iron (III) sulfate TS, dilute To 2 mL of ammonium iron (III) sulfate TS, add 1mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

Ammonium iron (III) sulfate 12-hydrate $FeNH_4(SO_4)_2 \cdot 12\text{-}H_2O$ [Special class].

Ammonium mercuric thiocyanate TS Dissolve 30 g of ammonium thiocyanate and 27 g of mercury (II) chloride in water to make 1000 mL.

Ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot H_2O$ [Special class].

Ammonium molybdate-sulfuric acid TS Dissolve 1.0 g of ammonium molybdate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

Ammonium molybdate TS Dissolve 21.2 g of ammonium molybdate in water to make 200 mL (10 %). Prepare before use.

Ammonium nickel (II) sulfate See ammonium nickel (II) sulfate hexahydrate.

Ammonium nitrate [Special class].

Ammonium oxalate See ammonium oxalate monohydrate.

Ammonium oxalate monohydrate $(NH_2)_2C_2O_4 \cdot H_2O$ [Special class].

Ammonium oxalate TS Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/ L).

Ammonium persulfate See ammonium peroxodisulfate.

Ammonium polysulfide TS $(NH_4)_2Sx$ [Ammonium Sulfide Solution (yellow), First class].

Ammonium pyrrolidinedithiocarbamate $C_5H_{12}N_2S_2$ [First class]

Ammonium pyrrolidinedithiocarbamate, Saturation Saturate Ammonium pyrrolidinedithiocarbamate (contains about 10 mg per L)

Ammonium sulfamate See ammonium amidosulfate.

Ammonium sulfamate TS See ammonium amidosulfate TS.

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 acd TS, shake, and filter, The pH of this solution is about 1.

Ammonium sulfide TS $(NH_4)_2S$ [Ammonium Sulfide Solution, (colorless), First class]. Store in small, well-filled containers, protected from light.

Ammonium tartrate See L-ammonium tartrate.

L-Ammonium tartrate $C_4H_{12}N_2O_6$ [L(+) Ammoniumtartrate, Special class].

Ammonium thiocyanate NH_4SCN [Special class].

Ammonium thiocyanate-cobaltous nitrate TS Dissolve 17.4 g of ammonium thiocyanate and 2.8 g of cobaltous (II) nitrate hexahydrate in water to make 100mL.

Ammonium thiocyanate TS Dissolve 8 g of ammonium thiocyanate in water to make 100mL (1 mol/L).

Ammonium vanadate (V) NH_4VO_3 [Special class].

Amphotericin B TS Dissolve 22.5 mg of amphotericin B in 9mL of sterilize purified water.

Amyl alcohol See n-Amyl alcohol

***n*-Amyl alcohol** $CH_3(CH_2)_4OH$, Clear, colorless liquid, having a characteristic odor. Sparingly soluble in water, and miscible with ethanol (95) or diethylether.
Refraction index n_D^{20} between 1.409 and 1.411
Specific gravity d_{20}^{20} between 0.810 and 0.820
Distilling range between 135 °C and 140 °C, not less than 95 vol %.

***t*-Amyl alcohol** $(CH_3)_2C(OH)CH_2CH_3$, Clear, colorless liquid, having a characteristic odor. Miscible with tert-butanol or 2-butanone, and freely soluble in water.
Specific gravity d_{20}^{20} between 0.808 and 0.815
Purity Acid and ester To 20 mL of t-amyl alcohol, add 20 mL of ethanol (95) and 5.0 mL of 0.1 mol/ L sodium hydroxide VS, and heat gently under a reflux condenser in a water-bath for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L hydrochloric acid VS. Perform a blank determination: not more than 1.25 mL of 0.1 mol/L sodium hydroxide VS is consumed.
Nonvolatile residue Evaporate 50 mL of t-amyl alcohol, and dry at 105 °C for 1 hour: the residue is not more than 1.6 mg.
Distilling range between 100 °C and 103 °C, not less than 95 vol %.

***tert*-Amyl alcohol** See t-amylalcohol.

Amyl alcohol, iso See 3-methyl-l-butanol.

Anhydrous caffeine See caffeine, anhydrous.

Anhydrous cupric sulfate See copper (II) sulfate (anhydrous).

Anhydrous dibasic sodium phosphate See disodium hydrogen phosphate.

Anhydrous dibasic sodium phosphate for pH determination See disodium hydrogen phosphate, for pH determination.

Anhydrous lactose $C_{12}H_{22}O_{11}$ [Same as the monograph Anhydrous Lactose].

Anhydrous potassium carbonate See potassium carbonate.

Anhydrous sodium acetate See sodium acetate, anhydrous.

Anhydrous sodium carbonate See sodium carbonate, anhydrous.

Anhydrous sodium sulfate See sodium sulfate, anhydrous

Aniline $C_6H_5NH_2$ [Special class].

***p*-Anisaldehyde** See 4-methoxybenzaldehyde.

***p*-Anisaldehyde-acetic acid TS** See 4-methoxybenzaldehyde-acetic acid TS.

***p*-Anisaldehyde-sulfuric acid TS** See 4-methoxybenzaldehyde-sulfuric acid TS.

***p*-Anisidine**

Anisole C_7H_8O [Special class]. A colorless liquid.
Boiling point : about 155 °C.
Specific gravity d_{20}^{20} : 0.995 ~ 1.001.

Anthrone $C_{14}H_{10}O$ [Special class]. Light yellow crystals or crystalline powder.
Melting point : 154 ~ 160 °C.
Preserve in a light-resistant tight container.

Anthrone TS Dissolve 35 mg of anthrone in 100mL of sulfuric acid.

Antimony (III) chloride $SbCl_3$ [Special class].

Antimony (III) chloride TS Wash chloroform with an equal volume of water twice or three times, add freshly ignited and cooled potassium carbonate, and allow to stand overnight in a well-closed container protected from light. Separate the chloroform layer, and distil it, preferably with protection from light. With this chloroform, wash the surface of antimony (III) chloride until the rinsing solution becomes clear, add the chloroform to this antimony trichloride to make a

saturated solution, and place in light-resistant, glass-stoppered bottles. Prepare before use.

Antimony trichloride See antimony (III) chloride.

Antimony trichloride TS See Antimony (III) chloride TS.

Antipyrine $C_{11}H_{12}N_2O$ [Same as the namesake monograph].

Aprotinin A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cattle. The pH is between 5.0 and 7.0

Content not less than 15000 KIE Units and not more than 25000 KIE Units of aprotinin permL.

Assay (i) *Trypsin solution* Mass an amount of crystallized trypsin equivalent to about 250 FIP Units of trypsin according to the labeled FIP Units, and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 10mL. Prepare before use, and preserve in ice. (ii) *Sample solution* Dilute a suitable quantity of aprotinin with sodium tetraborate-calcium chloride buffer silution, pH 8.0 so that eachmL of the solution contains 800 KIE Units of aprotinin, and use this solution as the sample solution. (iii) *Apparatus* Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver chloride electrode, a nitrogen-induction true and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at 25 ± 0.1 °C by means of a precise thermoregulator. (iv) *Procedure* To 5.0 mL of N-α-benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, and use this solutions as the substrate solution. Pipet 1mL of the trypsin solution, add sodium, tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution as the test solution I. Transfer 10.0 mL of the substrate solution to the reaction reservoir. adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 1mL of the test solution I previously allowd to stand at 25 ± 0.1 °C for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50 μL micropipette (minimum graduation of 1 μL), while stirring, to keep the reaction solution at pH 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcoum chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this siltuion as the test solution II. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add 1mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, previously allowd to stand at 25 ± 0.1 °C for 10 minutes, and perform a blank determination in the same manner. (v) *Calculation* Plot the amount of consumption(μL) of 0.1 mol/L sodum hydroxide VS against the reaction time(minutes), select linear reaction times, t_1 and t_2, designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v_1 and v_2, respectively, and designate μmol of sodium hydroxide consumed per minuteas as *M*.

$$M(\mu\text{mol NaOH/min}) = \frac{v_2 - v_2}{t_2 - t_1} \times \frac{1}{10} \times f$$

f: Factor of 0.1 mol/L sodium hydroxide VS

$$\text{KIE Units permL of aprotinin to be tested} = \frac{2(M_A - M_0) - (M_B - M_0)}{L} \times n \times 32.5$$

L: Amount (mL) of the sample solution added to the test solution Ⅱ

N: Dilution coefficient of aprotinin to be tested

M_A: μmol of sodium hydroxide consumed in 1 minute when the test solution Ⅰ is used

M_B: μmol of sodium hydroxide consumed in 1 minute when the test solution Ⅱ is used

M_0: μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

32.5: Equivalent coefficient for calculation of KIE Units from FIP Units

One KIE Unit means an amount of aprotinin making a reduction of 50 % off the potency of 2 Units of kalidinogenase at pH 8.0 and room temperature for 2 hours.

Storage Preserve in a light-resistant, hermetic container and in a cold place.

Aprotinin TS Measure 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 permL.

L-Arginine $C_6H_{14}N_4O_2$ White, crystals or crystalline powder. It has a characteristic odor.

Specific Optical Rotation $[\alpha]_D^{20}$ +26.0 - +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50mL, 200 mm).

Loss on drying not more than 0.5 % (1 g, 105 °C, 3 hours).

Content: not less than 98.0z and not more than 102.0z.

Assay. Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3mL of formic acid, add 50mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes to green through yellow (indicator: 10 drops of p-naphtholbenzein TS). Perform a blank determination in the same manner and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid VS

= 8.710 mg of $C_6H_{14}N_4O_2$

L-Arginine $C_6H_{14}N_4O_2$ White, crystals or crystalline powder. It has a characteristic odor.
Specific Optical Rotation $[\alpha]_D^{20}$ +26.0 - +27.9°(After drying, 4 g, 6 mol/L hydrochloric acid TS, 50mL, 200 mm).
Loss on drying not more than 0.5 % (1 g, 105 °C, 3 hours).

L-Arginine hydrochloride $C_6H_{14}N_4O_2 . HCl$ [Same as the name sake monograph]

Arsenazo III $C_{22}H_{18}As_2N_4O_{14}S_2$.

Arsenazo III TS Dissolve 0.1 g of arsenazo III in water to make 50 mL.

Arsenic (III) trioxide As_2O_3 [Arsenic (III) trioxide, Special class].

Arsenic (III) trioxide (standard reagent) [Diarsenic trioxide, Standard reagent for volumetric analysis].

Arsenic (III) trioxide TS Add 1 g of arsenic (III) trioxide to 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.

Ascorbic acid See L-ascorbic acid.

L-Ascorbic acid $C_6H_8O_6$ [L(+)-Ascorbic Acid, Special class].

Ascorbic acid for iron limit test See L-ascorbic acid.

0.02 g/dL Ascorbic acid-hydrochloric acid TS Dissolve 0.025 g of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

0.05 g/dL Ascorbic acid-hydrochloric acid TS Dissolve 0.05 g of L-ascorbic acid in 30 mL of methanol, add carefully hydrochloric acid to make 100 mL. Prepare before use.

L-Aspartic acid $C_4H_7O_4N$ [Special class].

Aspartic acid See L-aspartic acid.

Aspirin $C_9H_8C_4$ [Same as the name sake monograph].

A-type erythrocyte suspension Prepare a suspension containing 1 vol % of erythrocyte separated from human A-type blood in isotonic sodium chloride solution.

Balsam Canada balsam for microscopy. Before use, dilute to a suitable concentration with xylene.

Barbital $C_8H_{12}N_2O_3$ [Same as the namesake monograph].

Barbital buffer solution Dissolve 15 g of barbital sodium in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

Barbital sodium $C_8H_{11}N_2NaO_3$, White, odorless crystals of crystalline powder, having a bitter taste. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
pH The pH of a solution of barbital sodium (1 in 200) is between 9.9 and 10.3.
Loss on drying not more than 1.0 % (1 g, 105 °C, 4 hours).
Content not less than 98.5 %.
Assay Weigh accurately about 0.5 g of barbital sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10mL of dilute hydrochloric acid, and extract with 50mL of chloroform. Then extract with three 25 mL volumes of chloroform, combine the total extract, wash with two 5 mL volumes of water, and extract the washings with two 10mL volumes of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5 mL volumes 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 until the color of the solution changes from yellow to purple through light purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination in the same manner.

EachmL of 0.1 mol/L potassium hydroxide-ethanol VS
= 20.618 mg of $C_8H_{11}N_2NaO_3$

Barium chloride See barium chloride dihydrate.

Barium chloride dehydrate $BaCl_2 . 2H_2O$ [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 $Ba(OH)_2 . 8H_2O$ [Special class]. Store in tightly stoppered containers.

Barium hydroxide TS Saturate barium hydroxide octahydrate in freshly boiled and cooled water (0.25 mol/L). Prepare before use.

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].

Beclometasone dipropionate $C_{28}H_{37}ClO_7$ [Special class].

Beeswax [Same as the namesake monograph in part II, Yellow beeswax]

Benzaldehyde C_6H_5CHO [First class].

Benzalkonium chloride [Same as the namesake monograph}

Benzene C_6H_6 [Special class].

Benzidine $NH_2C_6H_4C_6H_4NH$ [Special class].

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 ~ 137 °C

***p*-Benzoquinone** $C_6H_4O_2$ Yellow to yellow-brown, crystals or rystalline powder, having a pungent odor. Soluble in ethanol (95) or diethyl ether, slightly soluble in water. It is gradually changed to a blackish brown color by light.
Melting point between 111 °C and 116 °C
Content not less than 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 sulfuricacid (1 in 15), dissolve 3 g of potassium iodide by shaking, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L sodium thiosulfate VS
= 5.405 mg of $C_6H_4O_2$

Benzyl benzoate $C_6H_5COOCH_2C_6H_5$ [Special class]. A colorless oily liquid. Congealing point: about 18 °C. Boiling point: about 323 °C.
Specific gravity d_{20}^{20}: 1.118~1.123.
Preserve in a light-resistant tight container.

Benzyl parahydroxybenzoate $C_{14}H_{12}O_3$, White, odorless, fine crystals or crystalline powder. Freely soluble in ethanol (95), acetone or diethyl ether, and very slightly soluble in water.
Melting point 109 - 112 °C
Residue on ignition not more than 0.1 %.
Content not less than 99.0 %.
Assay Proceed as directed in the Assay under Ethyl parahydroxybenzoate.

EachmL of 1 mol/L sodium hydroxide VS
= 228.25 mg of $C_{14}H_{12}O_3$

Berberine chloride $C_{20}H_{18}ClNO_4 \cdot xH_2O$ [Same as the name sake monograph].

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 between 7.0 and 7.5, then add water to make 975 mL, and again adjust to pH 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 not more than 20 minutes, then cool quickly, or sterilize fractionally on each of three successive days for 30 minutes at 100 °C.

Bilirubin $C_{33}H_{36}N_4O_5$

4,4'-Bis(diethylamino)benzophenone
$(C_2H_5)_2NC_6H_4]_2CO$ Light yellow crystals.
Content : not less than 98 %.
Assay Weigh accurately 0.25 g of 4,4'-bis(diethylamino)benzophenone, dissolve in 50mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner, and make any necessary correction.

EachmL 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 $Bi(NO_3)_3 . 5H_2O$ [Special class].

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 (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). To 20 mL of a mixture of solution A and solution B (1:1), add 80 mL of dilute sulfuric acid and 0.2 mL of hydrogen peroxide (30). Prepare before use.

Bismuth nitrate TS Dissolve 5.0 g of bismuth nitrate pentahydrate in acetic acid (100) to make 100 mL.

Bismuth potassium iodide TS Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, shake for 1 hour, add 20 mL of a solution of potassium iodide (2 in 5), shake thoroughly, allow to stand for 24 hours, filter, and designate this solution as solution A. Separately, dissolve 10 g of L-tartaric acid in 50 mL of water, add 5mL of solution A, and preserve in light-resistant, glass-stoppered bottles.

Bismuth sodium trioxide $NaBiO_3$ [Special class]. A yellow-brown powder.

Identification. (1) To 10 mg of bismuth sodium trioxide add 5 mL of a solution of manganese (II) nitrate hexahydrate (4 in 125) and 1 mL of diluted nitric acid (1 in 3), and shake vigorously for 10 seconds: a red-purple color is developed.
(2) Dissolve 10 mg of bismuth sodium trioxide in 2 mL of diluted hydrochloric acid (1 in 2): this solution responds to the Qualitative Tests (1) for sodium salt.

Bismuth subnitrate [Same as the namesake monograph].

Bismuth subnitrate TS Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, stir for 1 hour, then add 20 mL of a solution of potassium iodide (2 in 5), and shake well. After standing for 24 hours, filter, and preserve the filtrate in a light-resistant bottle.

Bis-trimethyl silyl acetamide $CH_3CON[Si(CH_3)_3]_2$, Colorless liquid
Refractive index n_D^{20} between 1.414 and 1.418
Specific gravity d_{20}^{20} between 0 825 and 0.835
Boiling point between 71 °C and 73 °C

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 in alkali hydroxides, and it does not dissolve in water, in ammonia TS, or in organic solvents.
Melting point not below 300 °C
Residue on ignition not more than 0.1 %.
Nitrogen content between 15.5 % and 16.5 %

Blue tetrazolium $C_{40}H_{32}Cl_2N_8O_2$, 3,3'-Dianisole-bis-[4,4'-(3,5-diphenyl) tetrazolium chloride]. Light yellow crystals. Freely soluble in methanol, inethanol (95) or chloroform, slightly soluble in water, and practically insoluble in acetone or ether.
Melting point about 245 °C (with decomposition).
Absorbance $E_{1\,cm}^{1\%}$ (252 nm) not less than 826 (methanol).

Blue tetrazolium TS, alkaline To 1 volume of a solution of blue tetrazolium in methanol (1 in 200), add 3 volumes of a solution of sodium hydroxide in methanol (3 in 25). Prepare before use.

Borane-pyridine complex C_5H_8BN
Content not less than 80 %.
Assay Accurately weigh about 30 mg of borane-pyridine complex, dissolve in 40 mL of 0.05 mol/L iodide solution, add 10 mL of diluted sulfuric acid (1 in 6), and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank deter-mination, and make any necessary correction.

EachmL of 0.1 mol/L sodium thiosulfate VS
= 1.549 mg of C_5H_8BN

Boric acid H_3BO_3 [Special class].

Boric acid-methanol buffer solution Weigh exactly 2.1 g of boric acid, dissolve in 28 mL of sodium hydroxide TS, and dilute with water to exactly 100 mL. Mix equal volumes of this solution and methanol, and shake.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS, add 43.90 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution, add 21.30 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.2 To 50 mL of 0.2 mol/L boric acid-0.2 mol/ L potassium chloride TS for buffer solution, add 26.70 mL of 0.2 mol/L sodium hydroxide VS and water to make 200mL.

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.

0.2 mol/L Boric acid-0.2 mol/L potassium chloride TS for buffer solution Dissolve 12.376 g of boric acid and 14.911 g of potassium chloride in water to make 1000 mL.

Boron trifluoride BF_3, Colorless gas, having a nirritating odor.
Melting point -127.1 °C.
Boiling point -100.3 °C.

Boron trifluoride-methanol TS A solution containing 14 w/v % of boron trifluoride (BF_3:67.81) in methanol.

Bovine serum albumin Obtained from cattle serum as Cohn's fifth fraction. Contains not less than 95 % of albumin.

Bovine serum albumin-isotonic sodium chloride solution Dissolve 0.1 g of bovine serum albumin in isotonic sodium chloride solution to make 100 mL. Prepare before use.

Brilliant green $C_{27}H_{34}N_2O_4S$, Fine, glistening, yellow crystals. It dissolves in water and in ethanol (95). The wavelength of absorption maximum: 623 nm.

Bromine Br [Special class].

Bromine-cyclohexane 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.

Bromine TS Prepare by saturating water with bromine as follows: Transfer 2 mL to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100mL of cold water, insert the stopper, and shake. Preserve in light-resistant containers, preferably in a cold place.

Bromocresol green $C_{21}H_{14}Br_4O_5S$ [Special class].

Bromocresol green-crystal violet TS Dissolve 0.3 g of bromocresol green and 0.075 g of crystal violet in 2 mL of ethanol (95), and dilute with acetone to make 100 mL.

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-sodium hydroxide-acetic acid-sodium acetate TS To 0.25 g of bromocresol green add 15 mL of water and 5 mL of dilute sodium hydroxide TS, then add a small quantity of acetic acid-sodium acetate buffer solution, pH 4.5, dissolve while shaking, and add acetic acid-sodium acetate buffer solution, pH 4.5, to make 500 mL. Wash 250 mL of the solution with two 100 mL volumes 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 VS and 20 mL ethanol (95), and add water to make 100 mL.
Purity To 0.2 mL of this TS, 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: not more than 0.2 mL is consumed.

Bromocresol green-sodium hydroxide TS Triturate 0.5 g of bromocresol green with 2.8 mL of 0.1 mol/L sodium hydroxide VS in a mortar, add water to make 200 mL, and filter, if necessary.

Bromocresol green TS Dissolve 0.05 g of bromocresol green in 100 mL of ethanol (95), and filter, if necessary.

Bromocresol purple $C_{21}H_{16}Br_2O_5S$ [Special class].

Bromocresol purple-dipotassium hydrogen 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 with three 60 mL volumes of chloroform.

Bromocresol purple-sodium hydroxide TS Triturate 0.4 g of bromocresol purple with 6.3 mL of dilute sodium hydroxide TS in a mortar, add water to make 250 mL, and filter, if necessary.

Bromocresol purple TS Dissolve 0.05 g of bromocresol purple in 100 mL of ethanol (95), and filter if necessary.

Bromophenol blue $C_{19}H_{10}Br_4O_5S$ [Special class].

Bromophenol blue-potassium biphthalate TS Dissolve 0.1 g of bromophenol blue in potassium biphthalate buffer solution, pH 4.6, to make 100mL.

Bromophenol blue TS Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol, and filter if necessary.

Bromophenol blue TS, dilute Dissolve 0.05 g of bromo-phenol blue in 100 mL of ethanol (99.5). Prepare before use.

Bromophenol blue TS, pH 7.0 Mix 10 mL of bromo-phenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with dilute sodium hydroxide TS.

***N*-Bromosuccinimide** $C_4H_4BrNO_2$.

***N*-Bromosuccinimide TS** Dissolve 1 g of N-bromosuc-cinimidein 1000 mL of water.

Bromothymol blue $C_{27}H_{28}Br_2O_5S$ [Special class].

Bromothymol blue-sodium hydroxide TS To 0.2 g of powdered bromothymol blue, add 5mL of dilute sodium hydroxide TS and a small quantity of water, dissolve by shaking in a water-bath at 50 °C, then add water to make 100 mL.

Bromothymol blue TS Dissolve 0.1 g of bromothymol blue in 100 mL of dilute ethanol, and filter, if necessary.

Brucin See brucin dihydrate.

Brucine dihydrate $C_{23}H_{26}N_2O_4 \cdot 2H_2O$ [Special class].

B-type erythrocyte suspension Prepare a suspension containing 1 vol % of erythrocyte separated from human B-type blood in isotonic sodium chloride solution.

***n*-Butanol** See 1-butanol.

1-Butanol $CH_3(CH_2)_2CH_2OH$ [Special class].

2-Butanol $CH_3CH_2CH(OH)CH_3$ [Special class].

2-Butanone $CH_3COC_2H_5$ [Special class].

Butyl acetate $C_6H_{12}O_2$, Clear, colourless liquid, flammable, slightly soluble in water and miscible with ethanol (95).

Specific gravity d_{20}^{20} about 0.88

Refractive index n_D^{20} about 1.395

Distilling range : Between 123 °C and 126 °C, not less than 95 vol %.

***n*-Butyl acetate** $CH_3COOCH_2CH_2CH_2CH_3$ [Special class].

***n*-Butylamine** $CH_3CH_2CH_2CH_2NH_2$, A colorless liquid, having an amine-like, characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. The solution in water shows alkalinity and rapidly absorbs carbon dioxide from the air.

Specific gravity d_{20}^{20} between 0.740 and 0.747

Distilling range: between 76.5 °C and 79 °C, not less than 96 vol %.

***n*-Butyl chloride** $CH_3(CH_2)_3Cl$, Clear and colorless liquid, miscible with ethanol (95) and with diethyl ether, practically in soluble in water. Boiling point: About 78 °C

Refractive index n_D^{20} between 1.401 and 1.045

Specific gravity d_{20}^{20} between 0.884 and 0.890

***n*-Butyl formate** $HCOO(CH_2)_3CH_3$, Clear and colorless liquid, having a characteristic odor.

Specific gravity d_{20}^{20} between 0.884 and 0.904

***tert*-Butylmethylether** $(CH_3)_3COCH_3$, Clear colorless liquid, having a specific odor.

Refractive index n_D^{20} 1.3689

Specific gravity d_{20}^{20} 0.7404

Butyl parahydroxybenzoate $HOC_6H_4COOCH_2CH_2CH_2CH_3$ [Same as the name sake monograph in part II, Butylparaben]

Butyrolactone $C_4H_6O_2$ Clear, colorless to practically colorless liquid.

Specific gravity d_4^{25} : between 1.128 and 1.135

Boiling point : 198 ~ 208 °C

Cadmium acetate See cadmium acetate dihydrate.

Cadmium acetate dihydrate $Cd(CH_3COO)_2 . 2H_2O$ [Special class].

White crystals or crystalline powder.

Identification. (1) Dissolve 0.2 g of cadmium acetate di-hydrate in 20 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL of iron (III) chloride TS: a red-brown color is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of sodium sulfide TS: a yellow precipitate is produced.

Cadmium ground metal Cd [First class].

Cadmium-ninhydrin TS Dissolve 0.05 g of cadmium acetate dihydrate in 5mL of water and 1 mL of acetic acid (100), add 2-butanone to make 50 mL, and dissolve 0.1 g of ninhydrin in this solution. Prepare before use.

Caffeine $C_8H_{10}N_4O_2 . H_2O$ [Same as the namesake monograph].

Caffeine, anhydrous $C_8H_{10}N_4O_2$ [Same as the namesake monograph].

Calcium carbonate $CaCO_3$ [Special class].

Calcium chloride See calcium chloride dihydrate.

Calcium chloride dihydrate $CaCl_2 . 2H_2O$ [Special class].

Calcium chloride for drying $CaCl_2$ [For drying].

Calcium chloride for Karl Fischer method $CaCl_2$ [For water determination].

Calcium chloride TS Dissolve 7.5 g of calcium chloride dihydrate in water to make 100 mL (0.5 mol/L).

Calcium hydroxide $Ca(OH)_2$ [First class].

Calcium hydroxide for pH determination Calcium hydroxide prepared for pH determination.

Calcium hydroxide pH standard solution See the pH Determination under the General Tests, Processes and Apparatus.

Calcium hydroxide TS To 3 g of calcium hydroxide add 1000 mL of cold distilled water, and occasionally shake the mixture vigorously for 1 hour. Allow to stand and use the supernatant liquid (0.04 mol/L).

Calcium nitrate See calcium nitrate tetrahydrate.

Calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot {}_4H_2O$[Special class].

Calcium oxide CaO [Special class].

Camphor $C_{10}H_{16}O$ [Same as the monograph *d*-Camphor or *dl*-Camphor].

***d*-Camphorsulfonic acid** $C_{10}H_{16}O_4S$, White crystals or crystalline powder, having a characteristic odor. Very 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 and colorless or pale yellow.
Loss on drying not more than 2.0 % (1 g 105 °C, 5 hours).
Content not less than 99.0 percent, calculated on the dried basis.
Assay Weigh accurately about 4 g of d-camphorsulfonic acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner.

EachmL 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, in chloroform, and in ether, and practically insoluble in water.
Melting point between 30 ~ 33 °C

Ethyl n-caprylate $C_{10}H_{20}O_2$ Clear and colorless to almost colorless liquid.
Specific gravity d_{20}^{20}: 0.864 . 0.871
Purity Related substances. Dissolve 0.1 g of ethyl ncaprylate in 10mL of dioxane and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 mL each of the sample solution and standard solution (1) as directed under Gas Chromatography according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak areas other than ethyl n-caprylate from the sample solution is not larger than the peak area of ethyl n-caprylate from the standard solution (1).

Operating conditions
Proceed the operating conditions in the Assay under Men-tha Oil except detection sensitivity and time span of measurement.
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). Adjust the detection sensitivity so that the peak area of ethyl n-caprylate obtained from 5 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of ethyl n-caprylate from 5 μL of the standard solution (1) is about 20 % of the full scale.
Time span of measurement: 3 times as long as the retention time of ethyl n-caprylate beginning after the solvent peak.

Carbazochrome $C_{10}H_{12}N_4O_3$ Yellow-red to red crystals or crystalline powder.
Melting point about 222 °C (with decomposition).
Content not less than 98.0 %.
Assay Dissolve about 0.2 g of carbazochrome, previously weighed accurately, in 20 mL of acetic acid (100) by heating, add 80 mL of acetic anhydride, cool, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

EachmL 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 to nearly white foliaceous or plate-like crystals or crystalline powder. Freely soluble in pyridine and in acetone, slightly soluble in ethanol (99.5), and practically insoluble in water. It readily sublimes when heated.
Melting point 243 . 245 °C
Purity Clarity and color of solution. To 0.5 g of carbazole add 20mL of ethanol (99.5), and dissolve by warming: the solution is clear.
Residue on ignition : Not more than 0.1 % (1 g).

Carbon dioxide CO_2 [Same as the namesake monograph].

Carbon monoxide CO, A toxic, colorless gas. Prepare by passing the gas generated by reacting formic acid with sulfuric acid through a layer of sodium hydroxide TS. Carbon monoxide from a metal cylinder may be used.

Carbon tetrachloride CCl_4 [Special class].

Carmofur $C_{11}H_{15}FN_3O_3$ [Same as the namesake monograph]

Casein, milk [Special class]. A white to light yellow powder or grain.
Identification. Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry : it exhibits absorption at the wave numbers of about 1650 cm^{-1}, 1540 cm^{-1} and 1250 cm^{-1}.

Casein peptone See peptone, casein.

Castor oil [Same as the namesake monograph in Part II].

Catechol $C_{11}H_{15}FN_3O_3$ [Special class].

Cefdinir lactam ring-cleavage lactones $C_{14}H_{15}N_5O_6S_2$ A White to yellow powder. A mixture of 4 diastereoisomers.
Identification Determine the infrared absorption spectrum of cefdinir lactam ring-cleavage lactones as directed in the paste method under the Infrared Spectrophotometry: it exhibits absorption at the wave num-

bers of about 1743 cm^{-1},1330 cm^{-1},1163 cm^{-1} and 1047 cm^{-1}.
Content not less than 90 %.
Assay Dissolve about 5 mg of cefdinir lactam ring-cleavage lactones in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed in the operating conditions of Purity(2) *Related substances* under Cefdinir, and calculate the areas of each peak by the automatic integration method. Determine the percentage of the total peak area of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

Cephaeline hydrobromate $C_{28}H_{38}N_2O_4 \cdot 2HBr \cdot xH_2O$, A white or light-yellow crystalline powder.
Purity Dissolve 0.01 g of cephaeline hydrobromate in 10 mL of the mobile phase, and use this solution as the test solution. Pipet 1mL of the test 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 according to the operating conditions in the Component determination under Ipecac: the total peak area of peaks other than cephaeline from the test solution is not larger than the peak area of cephaeline from the standard solution.

Cerium (III) nitrate hexahydrate $Ce(NO_3)_3 \cdot 6H_2O$, A colorless or light yellow, crystalline powder. It dissolves in water.
Purity (1) Chloride: not more than 0.036 %. (2) Sulfate: not more than 0.120 %.
Content: not less than 98.0 %.
Assay To about 1.5 g of cerous nitrate, accurately weighed, add 5 mL of sulfuric acid, and heat it until white fumes are evolved vigorously. After cooling, add 200 mL of water, 0.5 mL of 0.1 mol/L silver nitrate VS, dissolve 5 g of ammonium persulfate, dissolve, and boil it for 15 minutes. After cooling, add 2 drops of 1,10-phenanthroline TS, and titrate with 0.1 mol/L ferrous ammonium sulfate VS until the pale blue color of the solution changes to red.

EachmL 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 1000mL.

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 160mL of diluted dilute nitric acid (9 in 50). Use within 3 days.

Cerium sulfate $Ce(SO_4)_2$ [special class]

Cerous nitrate See cerium (III) nitrate hexahydrate.

Cerous nitrate TS See cerium (III) nitrate TS.

Cetanol [Same as the namesake monograph in Part II].

Cetrimide $C_{17}H_{38}Br$, White to pale yellowish white powder, havinga faint, characteristic odor.
Purity Clarity of solution Dissolve 1.0 g of cetrimide in 5 mL of water: the solution is clear.
Content : not less than 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 VS and 10 mL of a freshly prepared solution of potassium iodide (1 in 20), shake well, allow to stand and remove the chloroform layer. Wash the solution with three 10mL volumes 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 deep brown color of the solution almost disappears, add 2 mL of chloroform, and titrate again until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the chloroform layer is decolorized. Perform a blank determination with 20 mL of water, 10 mL of a solution of potassium iodide (1 in 20) and 40 mL of hydrochloric acid.

EachmL of 0.05 mol/L potassium iodate VS
= 33.640 mg of $C_{17}H_{38}Br$

Chenodeoxycholic acid for thin-layer chromatography $C_{24}H_{40}O_4$, White crystals or crystalline powder. Very soluble in methanol or aceticacid (100), freely soluble in ethanol (95), soluble in acetone, sparingly soluble in ethyl acetate, slightly soluble in chloroform, and practically in soluble in water.
Melting point About 119 °C (recrystallize from ethyl acetate).
Purity Related substances Dissolve 0.025 g of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (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: any spot other than the principal spot at the R_f value of about 0.4 does not appear.
Content not less than 98.0 %.
Assay Weigh accurately about 0.5 g of chenodeoxycholic acid for thin-layer chromatography, previously dried under reduced pressure (phosphorus (V) oxide) at 80 °C for 4 hours, and dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L sodium hydroxide VS. Near the end point, add 100mL of freshly boiled and cooled water, and titrate again.

EachmL of 0.1 mol/L sodium hydroxide VS
= 39.258 mg of $C_{24}H_{40}O_4$

Chloral hydrate $CCl_3CH(OH)_2$ [First class].

Chloral hydrate TS Dissolve 5 g of chloral hydrate in 3 mL of water.

Chloramine See sodium toluensulfonchloramide trihydrate.

Chloramine TS See sodium toluensulfonchloramide TS.

Chloramphenicol $C_{11}H_{12}C_{12}N_2O_5$ [Same as the monograph Chloramphenicol].

Chlorauric acid See hydrogen tetrachloroaurate (III) tetrahydrate.

Chlorauric acid TS See hydrogen tetrachloroaurate (III) tetrahydrate TS.

Chlorinated lime [Same as the namesake monograph in Part II].

Chlorinated lime TS Triturate 1 g of chlorinated lime with 9 mL of water, and filter. Prepare before use.

Chlorine Cl_2 A yellow-green gas, having a suffocating odor. It is heavier than air, and dissolves in water. Prepare from chlorinated lime with hydrochloric acid. Chlorine from a metal cylinder may be used.

Chlorine TS Use a saturated solution of chlorine in water. Preserve this solution in fully filled, light-resistant, glass-stoppered bottles, preferably in a cold place.

***p*-Chloroaniline** See 4-chloroaniline.

4-Chloroaniline $H_2NC_6H_4Cl$, White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in hot water.
Melting point between 70 °C and 72 °C
Residue on ignition not more than 0.10 % (1 g).

***p*-Chlorobenzene sulfonamide** See 4-chlorobenzene sulfonamide.

4-Chlorobenzene sulfonamide $ClC_6H_4SO_2NH_2$, White to pale yellow, odorless, crystalline powder. Dissolves in acetone.
Purity Related substances Dissolve 0.60 g of 4-chlorobenzene sulfonamide in acetone to make exactly 300mL, and perform the test with 5 μL of this solution as directed in the Purity (5) under Chlorpropamide: any spot other than the principal spot at the Rf value of about 0.5 does not appear.

***p*-Chlorobenzoic acid** See 4-chlorobenzoic acid.

4-Chlorobenzoic acid ClC_6H_4COOH, White crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically ins oluble in water.
Melting point between 238 °C and 242 °C
Content not less than 99.0 %.
Assay Mass accurately about 0.3 g of 4-chlorobenzoic acid, dissolve in 30mL of neutralized ethanol, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

EachmL of 0.1 mol/L sodium hydroxide VS
= 15.657 mg of $C_7H_5ClO_2$

Chlorobutanol $Cl_3CC(CH_3)_2OH$ [Same as the namesake monograph]

Chloroform $CHC1_3$ [Special class].

Chloroform, ethanol-free Mix 20 mL of chloroform with 20 mL of water, gently shake for 3 minutes, separate the chloroform layer, wash the layer again with two 20 mL volumes of water, and filter it through dry filter paper. To the filtrate, add 5 g of anhydrous sodium sulfate, shake for 5 minutes, allow the mixture to stand for 2 hours, and filter through dry filter paper. Prepare before use.

***p*-Chlorophenol** See 4-Chlorophenol.

4-Chlorophenol ClC_6H_4OH, Colorless or pale red crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol (95), chloroform, diethyl ether or glycerin, and sparingly soluble in water.
Melting point about 43 °C
Content: not less than 99.0 %.
Assay Weigh accurately about 0.2 g of 4-chlorophenol, and dissolve in water to make 100mL. Measure exactly 25 mL of this solution into an iodine flask, add exactly 20 mL of 0.05 mol/L bromine VS and then 5mL of hydrochloric acid, stopper immediately, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5mL of a solution of potassium iodide (1 in 5), stopper immediately, shake well, and titrate with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

EachmL of 0.05 mol/L bromine VS
= 3.2140 mg of C_6H_5ClO

Preserve in tight, light-resistant containers.

Chloroplatinic acid See hydrogen hexachloroplatinate (IV) hexahydrate.

Chloroplatinic acid-potassium iodide TS See hydrogen hexachloroplatinate (IV)-potassium iodide TS.

Chloroplatinic acid TS See hydrogen hexachloroplatinate(IV) TS

Cholesterol $C_{27}H_{45}OH$ [Same as the namesake monograph in Part II].

Choline chloride $[(CH_3)_3NCH_2CH_2OH]\cdot Cl$, A white crystalline powder.

Chromium trioxide See chromium (IV) trioxide.

Chromium trioxide TS See chromium (IV) trioxide TS.

Chromotropic acid See disodium chromotropate dihydrate.

Chromotropic acid TS Dissolve 0.05 g of disodium chromotropate dihydrate in the solution prepared by cautiously adding 68 mL of sulfuric acid to 30 mL of water, cooling, then adding water to make 100 mL. Preserve in light-resistant containers.

Chromotropic acid TS, concentrated Suspend 0.5 g of disodium chromotropate dihydrate in 50mL of sulfuric acid, centrifuge, and use the supernatant liquid. Prepare before use.

Cinchonidine $C_{19}H_{22}N_2O$, White crystals or crystalline powder. Soluble in ethanol (95), methanol or chloroform, sparingly soluble in diethyl ether, and practically in soluble in water. A solution of cinchonidine in ethanol (95) (1 in 100) is levorotatory.
Melting point about 207 °C
Content not less than 98.0 %.
Assay Weigh accurately about 0.3 g of cinchonidine, dissolve in 20 mL of acetic acid (100), add 80mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner, and make necessary correction.

EachmL of 0.1 mol/L perchloric acid VS
= 14.720 mg Of $C_{19}H_{22}N_2O$

Citric acid See citric acid monohydrate.

Citric acid (100) $C_6H_8O_7$ [Special class].

Citric acid-acetic acid TS To 1 g of citric acid monohy-drate, add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve under shaking.

Citric acid monohydrate $C_6H_8O_7.H_2O$ [same as the namesake monograph].

0.1 mol/L Citric acid TS Dissolve 2.1 g of citric acid monohydrate in water to make 1000mL.

1 mol/L Citric acid TS for buffer solution Dissolve 210.14 g of citric acid monohydrate in water to make 1000mL.

Clotrimazole $C_{22}H_{17}ClN_2$ [Same as the name sake monograph].

Cloxazolam $C_{17}H_{14}Cl_2N_2O_2$ [Same as the namesake monograph].

Cobaltous chloride See cobaltous (II) chloride hexahydrate.

Cobaltous chloride-ethanol TS Dissolve 0.5 g of cobaltous (II) chloride hexahydrate, previously dried at 105 °C for 2 hours, in ethanol (99.5) to make 100 mL.

Cobaltous chloride TS Dissolve 2 g of cobaltous (II) chloride hexahydrate in 1 mL of hydrochloric acid and water to make 100 mL (0.08 mol/L).

Cobaltous (II) nitrate hexahydrate $Co(NO_3)_2\cdot 6H_2O$ [Special class].

Cobaltous nitrate See cobaltous (II) nitrate hexahydrate.

Concentrated chromotropic acid TS See chromotropic acid TS, concentrated.

Concentrated diazobenzenesulfonic acid TS See diazo-benzenesulfonic acid TS, concentrated.

Copper Cu [Special class].

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 solution (28): a dark blue color is developed.

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.

Copper (II) chloride-aceton TS Dissolve 0.3 g of copper (II) chloride dihydrate in aceton to make 10 mL.

Copper (II) chloride dihyrate $CuCl_2.2H_2O$ [Special class].

Copper (II) disodium ethylenediamine tetraacetate tetrahydrate $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$ A blue powder.
pH between 7.0 and 9.0
Purity Clarity and color of solution Add 0.10 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate to 10 mL of freshly boiled and cooled water: the solution is blue in color and clear.
Content: not less than 98.0 %.
Assay Weigh accurately about 0.45 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate, and add water to make exactly 100mL. Pipet 10 mL of this solution, adjust the pH of the mixture to about 1.5 by adding 100 mL of water, and dilute with nitric acid, then add 5mL of a solution of 1,10-phenanthroline monohy-drate in methanol (1 in 20), and titrate with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

EachmL of 0.01 mol/L bismuth nitrate VS
= 4.698 mg of $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$

Copper (II) hydroxide $Cu(OH)_2$, Light blue powder. Practically insoluble in water.
Content not less than 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 a solution of ammonium chloride (3 in 50), 3 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator, and titrate with 0.01 mol/L disodium ethylenedia-minetetraacetate VS until the color of the liquid is changed from yellow-green to red-purple.

EachmL of 0.01 mol/L disodium
ethylenediaminetetraacetate VS
= 0.9756 mg of $Cu(OH)_2$

Copper (II) sulfate (anhydrous) $CuSO_4$ [First class].

Copper (II) sulfate pentahydrate $CuSO_4 \cdot 5H_2O$ [Special class].

Copper (II) sulfate-pyridine TS Dissolve 4 g of copper(II) sulfate pentahydrate in 90mL of water, then add 30 mL of pyridine, Prepare before use.

Copper (II) sulfate solution, alkaline Dissolve 150 g of potassium bicarbonate, 101.4 g of potassium carbonate and 6.93 g of copper(II) sulfate pentahydrate in water to make 1000mL

Copper (II) sulfate TS Dissolve 12.5 g of copper (II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L)

Copper (Standard reagent) Cu [Standard reagent for quantitative analysis].

Copper TS, Alkaline Dissolve 70.6 g of disodiumhydrogen phosphate 12-hydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600mL of water, and add 20mL of a solution of sodium hydroxide (1 in 5). To this mixture add, with stirring, 100 mL of a solution of cupric sulfate (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate VS and water to make 1000 mL.

Corn oil [Same as the namesake monograph in Part II].

Cortisone acetate $C_{23}H_{30}O_6$ [Same as the namesake monograph].

Cottonseed oil A refined, nonvolatile fatty oil obtained from the seed of plants of Gossypium hirsutum Linne (Gossypium) or of other similar species. A pale yellow, odorless, oily liquid. Miscible with ether, or hexane. Slightly soluble in ethanol (95).
Refractive index n_D^{20} between 1.472 and 1.474.
Specific gravity d_{20}^{20} between 0.915 and 0.921.
Acid value not more than 0.5.
Saponification value between 190 and 198.
Iodine value between 103 and 116.

Cresol $CH_3C_6H_4(OH)$ [Same as the namesake monographin Part II].

m-Cresol $CH_3C_6H_4(OH)$ [Special class].

Cresol red $C_{21}H_{18}O_5S$ [Special class].

Cresol red TS Dissolve 0.1 g of cresol red in 100mL of ethanol (95), and filter, if necessary.

Crystallized trypsin To trypsin obtained from bovine pancreas gland add an appropriate amount of trichloroacetic acid to precipitate the trypsin, and recrystallize in ethanol(95). White to yellowish white crystals or powder. It is odorless. Freely soluble in water and in sodium, tetraborate-calcium chloride buffer solution, pH 8.0.
Content not less than 45 FIP Units of trypsin per mg.

Crystal violet $C_{25}H_{30}ClN_3 \cdot 9H_2O$ [Special class].

Crystal violet TS Dissolve 0.1 g of crystal violet 10mL of glacial acetic acid.

Cu-PAN Prepare by mixing 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) with 11.1 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate. A grayish orange-yellow, grayish red-brown or light grayish purple powder.

Absorbance Dissolve 0.50 g of Cu-PAN in diluted 1,4-dioxane (1 in 2) to make exactly 50mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL. Read the absorbance of this solution at 470 nm as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank solution: the absorbance is not less than 0.48.
Purity Clarity and color of solution Dissolve 0.50 g of Cu-PAN in 50 mL of diluted 1,4-dioxane (1 in 2): the solution is clear and yellow-brown.

Cu-PAN TS Dissolve 1 g of Cu-PAN in 100 mL of diluted 1,4-dioxane (1 in 2).

Cupferron $C_6H_9N_3O_2$ [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, strong See copper (II) acetate monohydrate TS, strong.

Cupric carbonate See cupric carbonate monohydrate.

Cupric carbonate monohydrate $CuCO_3 . Cu(OH)_2 . H_2O$, A blue to blue-green powder. It is insoluble in water, and dissolves foamingly in diluteacid. It dissolves in ammonia TS and shows a deep blue color.
Purity (1) Chloride not more than 0.036 %.
(2) Sulfate not more than 0.120 %.
(3) Iron Dissolve 5.0 g of cupric carbonate in excess ammonia TS and filter. Wash the residue with ammonia TS, dissolve in dilute hydrochloric acid, add excess ammonia TS and filter. Wash the residue with ammonia TS, and dry to constant mass: the residue is not more than 10 mg.

Cupric chloride See copper (II) chloride dihydrate.

Cupric chloride-aceton TS See copper (II) chloride-aceton TS.

Cupric hydroxide See Copper (II) hydroxide

Cupric sulfate See copper (II) sulfate pentahydrate.

Cupric sulfate TS See copper (II) sulfate TS.

1 mol/L Cupriethylenediamine TS Put 100 g of copper (II) hydroxide in a 1 L thick-walled bottle marked a 500 mL line, and add water to make 500 mL. Connect the bottle with a liquid introducing funnel, a nitrogen introducing glass tube and a gas removing glass tube. Adjust so that the lower end of the nitrogen introducing tube is located at about 1.3 cm above of the bottom of the bottle. Introduce the nitrogen for about 3 hours to replacing the inside gas by adjusting the pressure (about 14 kPa) to get a mild bubbling. Then add gradually 160 mL of ethylenediamine TS through the funnel while introducing the nitrogen and cooling the bottle with the running water, and replace the funnel with a glass rod to close tightly. After introducing the nitrogen for further 10 minutes replace the gas removing tube with a glass rod to close tightly. Keep the inside pressure with the nitrogen to about 14 kPa. After allowing the bottle to stand for about 16 hours while occasional shaking filter the content if necessary using a glass-filter under reducing pressure, and reserve under nitrogen atmosphere. The concentration of copper (II) ion of this solution is about 1.3 mol/L. Determine the concentration of ethylenediamine of this solution, X (mol/L) and copper(II), ion Y (mol/L) by the following Assays, and adjust to that X is 1.96 to 2.04, Y is 0.98 to 1.02 and X/Y is between 1.96 and 2.04 by adding water, copper(II) hydroxide or ethylene diamine TS, then determine X and Y again in the same manner, and use this solution as the test solution.
Assay (1) *Ethylenediamine* Pipet 1 mL ($V1$) of the solution to be assayed, add 60 mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (pH Determination method; endpoint is about pH8.4).

$$X = \frac{N_1 a}{V_1}$$

X: Concentration of ethylenediamine (mol/L),
a: Volume of 0.1 mol/L hydrochloric acid VS consumed for the titration (mL), and
N_1: Concentration of 0.1 mol/L hydrochloric acid VS (mol/L), and

(2) *Copper (II) ion* Pipe 2 mL (V2) of the solution to be assayed, add 20 mL of water, about 3 g of potassium iodide and 50 mL of diluted sulfuric acid (1 to 9), shake for 5 minutes, and titrate the liberated iodine with 0.1 mol/L sodium thio sulfate VS. When the solution turns yellow at near the end point, add 3 mL of starch TS and 10 mL of a solution of ammonium thiocyanate (2 in 10), and the ntitrate until the blue color disappears.

$$Y = \frac{N_2 b}{V_2}$$

Y: Concentration of copper (II) ion (mol/L),
b: Volume of 0.1 mol/L sodium thiosulfate VS consumed for the titration (mL), and
N_2. Concentration of 0.1 mol/L sodium thiosulfate VS (mol/L).

Curcumin $C_{21}H_{20}O_6$ [Special class].

Curcumin TS Dissolve O.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare before use.

Cyanoacetic acid $C_3H_3NO_2$, White to light yellow crystals. Very soluble in water.
Content not less than 99 %.
Assay Weigh accurately about 300 mg of cyanoacetic acid, add 25 mL of water and 25 mL of ethanol (95) to dissolve, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide VS
= 85.06 mg of $C_3H_3NO_2$

Cyanogen bromide TS To 100mL of ice-cold water, add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide TS drop-wise until the color of bromine just disappears. Prepare this test solution in a hood before use. On handling this solution, be careful not to inhale its vapors, which are very toxic.

Cyanogen bromide TS for thiamine assay To 100mL of ice-cold water, add 2 mL of bromine, shake vigorously, and add ice-cold potassium thiocyanate TS drop-wise until the color of bromine just disappears. Prepare this test solution in a hood, and preserve in a cold place. Use within 1 month. On handling this test solution, be careful not to inhale its vapors, which are very toxic.

Cyanogen chloride TS To 60 mL of ethanol (95), add 3.4 mL of acetic acid (100), 10 mL of sodium cyanide TS and 25 mL of a solution of sodium toluensulfonchloramide trihydrate (7 in 50), while cooling with ice, shake, and then add water to make 100mL. Use within 1 hour.

Cyclohexane C_6H_{12} [Special class].

3-Cyclohexypropionic acid $C_9H_{16}O_2$ Clear liquid.
Refractive index n_D^{20} about 1.4648
Boiling point about 130 °C
Specific gravity d_{20}^{20} about 0.998.

Cycloserine of reaction TS Mix of equal parts of sodium pentacyanonitrosylferrate (III) dehydrate (1 in 05) and sodium hydroxide (4 in 25). Preserve in brown bottle, and use within 24 hours.

L-Cysteine hydrochloride See L-cysteine hydrochloride monohydrate.

L-Cysteine hydrochloride monohydrate $HSCH_2CH(NH_2)COOH \cdot H_2O$ [Special class].

L-Cystine
$HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$ [L-(-)Cystine, Special class].

***N*-Demethylerythromycin** $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

***N*-Demethylroxithromycin** $C_{40}H_{74}N_2O_{15}$ White powder.
Identification : Determine the infrared absorption spectrum of a solution of the substance to be tested in chloroform (1 in 20) as directed in the solution method under Infrared Spectrophotometry using a 0.1 mm cell made of potassium bromide: 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}.

2'-Deoxyuridine for liquid chromatography see 2'-Deoxyuridine, for liquid chromatography

Diacetyl $CH_3COCOCH_3$, A yellow to yellow-green, clear liquid, having a strong, pungent odor. Miscible with ethanol (95) and with diethyl ether, and freely soluble in water.
Congealing point between -2.0 °C and -5.5 °C.
Refractive index n_D^{20} between 1.390 and 1.398.
Specific gravity d_{20}^{20} between 0.98 and 1.00.
Boiling point between 85 °C and 91 °C
Purity Clarity of solution Dissolve 1.0 g of diacetyl in 10mL of water: the solution is clear.
Content not less than 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 until the color of the solution changes from blue to yellow-green through green (indicator: 3 drops of bromophenol blue TS). Perform a blank determination in the same manner, make any necessary correction.

EachmL of 0.5 mol/L hydrochloric acid VS
= 21.523 mg of $C_4H_6O_2$

Diacetyl TS Dissolve 1mL of diacetyl in water to make 100 mL, and dilute 5mL of this solution with water to make 100 mL. Prepare before use.

2,3-Diaminonaphthalene $C_{10}H_{10}N_2$ Light yellow-brown crystals or powder. Slightly soluble in ethanol (95) or diethyl ether, and practically insoluble in water.
Melting point between193 °C and 198 °C.
Sensitivity Pipet separately 40 mL each of the selenium standard solution and diluted nitric acid (1 in 60) as the blank solution into beakers, and to these solutions add ammonia solution (28) to adjust the pH to between 1.8 and 2.2. Dissolve 0.2 g of hydroxylammonium chloride in each of these solutions under gentle shaking, add 5 mL of 2,3-di-aminonaphthalene TS, mix by shaking, and allow to stand for 100 minutes. Transfer these solutions to separators separately, rinse the beakers with 10 mL of water, add these rinsings to the separators, extract each with 5.0 mL of cyclo-hexane by thorough shaking for 2 minutes, and centrifuge the cyclohexane layers to remove moisture. When the ab-

sorbance at 378 nm of cyclohexane extract obtained from selenium standard solution is determined using the solution obtained from the blank solution as the reference solution as directed under the Ultraviolet-visible Spectrophotometry: it is not less than 0.08.
Selenium standard solution - Weigh accurately 0.040 g of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), by heating on water-bath if necessary, and add water to make exactly 1000 mL. Pipet 5mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, and add diluted nitric acid (1 in 60) to make exactly 50 mL. Prepare before use. This solution contains 0.04 μg of selenium (Se) permL.

2,3-Diaminonaphthalene TS Dissolve 0.10 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylammonium chloride in 0.1 mol/L hydrochloric acid TS to make 100 mL.

2,4-Diaminophenol hydrochloride $C_6H_8N_2O \cdot 2HCl$ Pale yellow-brown to grayish yellow-green crystalline powder. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
Purity Clarity of solution Dissolve 1.0 g of 2,4-diaminophenol hydrochloride in 20 mL of water: the solution is clear or a slight turbidity is produced.
Loss on drying not more than 0.5 % (1 g, 105 °C, 3 hours).
Residue on ignition not more than 0.5 % (1 g).
Content not less than 98.0 %.
Assay Weigh accurately about 0.2 g of 2,4-diaminophenol hydrochloride, dissolve in 50mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L silver nitrate VS
= 9.853 mg of $C_6H_8N_2O \cdot 2HCl$

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.

Diazobenzenesulfonic acid TS Weigh 0.9 g of sulfanilic acid, previously dried at 105 °C for 3 hours, dissolve it in 10 mL of dilute hydrochloric acid by heating, and add water to make 100mL. Pipet 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 °C for 3 hours, dissolve it 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) drop-wise under stirring. Allow to stand in ice water for 10 minutes, and add 1 mL of a solution of sulfaminic acid (1 in 20). Prepare before use.

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 dilute the filtrate with water to make exactly 100 mL. Pipet 1.5 mL of this solution, cool in an ice-bath, and add exactly 1 mL of sodium nitrite solution (1 in 20) dropwise, while shaking. Cool in an ice-bath for 10 minutes, add cold water to make exactly 50mL. Store in a cold place, and use within 8 hours.

N,N'-Dibenzylethylenediamine diacetate A white to slightly pale yellow crystalline powder.
Identification. Determine the infrared absorption spectrum of the substance to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry : 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. not less than 99.0 %.
Assay. Weigh accurately about 25 mg of N,N'-dibenzylethylene diamine diacetate, dissolve in 25mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate, anhydrous 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 the solution containing 1.02 g of 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 sample solution. Separately, weigh accurately about 8 mg of acetic acid(100),
add 25mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate, anhydrous 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 control solution.
Perform the test with exactly 20 mL each of the sample solution and control solution as directed under Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. After making correction for the peak areas based on the valiance of the base-line and the peak of acetic acid on the chromatogram obtained with the sample solution, calculate the amount of N,N'-dibenzylethylenediamine by the area percentage method.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with

octadecylsilanized silicagel for liquid chromatography (5 mm in particle diameter).

Column temperature: A constant temperature of about 40 °C.

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 is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of N,N'-dibenzylethylenediamine.

System suitability

System performance: Dissolve an amount of Benzyl penicillin Benzathine, equivalent to about 85,000 Units, in 25 mL of methanol, add a solution containing 1.02 g of 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 the solution containing 1.02 g of 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. When the procedure is run with 20 mL of this solution under the above operating conditions, N,N'-dibenzylethylenediamine and benzylpenicillin are eluted in this order with there solution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of N,N'-dibenzylethylenediamine is not more than 2.0 %.

2,6-Dibromo-N-chloro-1,4-benzoquinonemonoimine $C_6H_2Br_2 \cdot ClNO$ [Special class].

2,6-Dibromo-N-chloro-1,4-benzoquinone monoamine TS Dissolve 0.5 g of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in methanol to make 100mL.

2,6-Dibromoquinone chlorimide See 2,6-dibromo-N-chloro-l, 4-benzoquinone monoimine.

2,6-Dibromoquinone chlorimide TS See 2,6-dibromo-N-chloro-l, 4-benzoquinone monoimine.

Dibucaine hydrochloride $C_{20}H_{29}N_3O_2 \cdot HCl$ [Same as the namesake monograph].

Dibutyl ether $C_8H_{18}O$ A Clear, colorless liquid. Water non miscible liquid.

Specific gravity d_{20}^{20} between 0.798 and 0.771.

1,2-Dichloroethane $ClCH_2CH_2Cl$ [Special class].

Dichlorofluorescein $C_{20}H_{10}Cl_2O_5$ [Special class]. Orange to red-brown powder.

Identification (1) Dissolve 0.1 g in 10mL of sodium hydroxide TS: the solution is an orange-red color, and redorange precipitates appear by the addition of 10 mL of dilute hydrochloric acid.

(2) Dissolve 0.1 g in 10mL of sodium hydroxide TS, and add 40 mL of water: a green-yellow fluorescence is exhibited.

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 VS, and dilute with water to make 100 mL.

2,6-Dichloroindophenol sodium dehydrate $C_{12}H_6CI_2NNaO_2 \cdot 2H_2O$ [Special class].

2,6-Dichloroindophenol sodium TS Add 0.1 g of 2,6-dichloroindophenol sodium dihydrate to 100 mL of water, warm, and filter. Use within 3 days.

2,6-Dichloroindophenol sodium TS for titration See the monograph Ascorbic Acid Powder.

Dichloromethane CH_2Cl_2 [Special class].

2,6-Dichlorophenol-indophenol sodium See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-dichloroindophenol sodium TS.

2,6-Dichlorophenol-iadophenol sodium TS for titration See 2,6-dichloroindophenol sodium TS for titration.

N,N'-Dicyclohexylcarbodiimide
$C_6H_{11}N=C=NC_6H_{11}$ Colorless or white crystals or crystalline mass. Dissolves in ethanol (95), but decomposes in water to produce a white precipitate.
Melting point between35 °C and 36 °C

N,N'-Dicyclohexylcarbodiimide-dehydrated 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) tomake100 mL.
Storage Preserve in tight containers, in a cold place.

Dicyclohexyl phthalate $C_6H_4(COOC_6H_{11})_2$, A white, crystalline powder.
Melting point between63 °C and 66 °C
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.

Diethanolamine $C_4H_{11}NO_2$, A colorless, viscous liquid.
Melting point between 27 °C and 30 °C
Water Less than 0.1 %

Diethanolamine hydrochloride $C_4H_{11}NO_2$ ・ HC,l A pale yellow liquid.

Refractive index n_D^{20} between 1.515 and 1.519.

Specific gravity d_{20}^{20} between 1,259 and 1.263.

*Wate*r Less than 0.1 %

Diethylamine $(C_2H_5)_2NH$, A clear, colorless liquid, having an amine-like odor. Miscible with water or ethanol (95). The solution in water is alkaline, and readily absorbs carbon dioxide in air.

Specific gravity d_{20}^{20} between 0.702 and 0.708.

Distilling range between 54 °C and 58 °C; not less than 96 vol %.

Content not less than 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 VS, and titrate the excess of sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination, and make any necessary correction.

EachmL of 0.5 mol/L sulfuric acid VS
= 73.14 mg of $(C_2H_5)_2NH$

Diethylene glycol $HO(CH_2CH_2O)_2H$, Colorless and odorless liquid. Miscible with water or ethanol (95).

Specific gravity d_{20}^{20} between 1.118 and 1.120.

Diethylene glycol dimethyl ether $(CH_3OCH_2CH_2)_2O$, Clear and colorless liquid, miscible with water.

Specific gravity d_{20}^{20} between 0.940 and 0.950.

Distilling range between 158 °C and 160 °C, not less than 95 vol %.

Diethylene glycol monoethyl ether $C_2H_5(OCH_2CH_2)_2OH$ [2-(2-ethoxyethoxy)ethanol], A clear, colorless liquid, of which boiling point is about 203 °C. It freely mix with water.

Refractive index n_D^{20} between 1.425 and 1.429.

Specific gravity d_{20}^{20} between 0.990 and 0.995.

Acid (as CH_3COOH) Less than 0.01 %.

Diethyl ether see ether

N,N-Diethyl-N'-1-naphthylethylenediamine oxalate $C_{18}H_{24}N_2O_4$ white crystalline powder.

Identification. Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry : it exhibits absorption at the wave numbers 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 of solution. To 0.1 g add 20 mL of water, and dissolve by warming: the solution is clear.

N,N-Diethyl-N'-1-naphthylethylenediamineoxalate-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.

N,N-Diethyl-N'-1-naphthylethylenediamine oxalate TS Dissolve 1 g of N,N-Diethyl-N'-1-naphthylethylenediamine oxalate in water to make 1000mL.

Digitonin $C_{56}H_{92}O_{29}$ White to whitish crystals or crystalline powder.

Optical rotation $[\alpha]_D^{20}$: −47 ~ −50 (2 g dried at 105 °C for 2 hours, diluted acetic acid (100) (3 in 4), 50 mL, 100 mm).

Sensitivity : Dissolve 0.5 g of digitonin in 20mL of ethanol (95) by warming, and add ethanol (95) to make 50 mL. To 0.5 mL of this solution add 10mL of a solution of cholesterol in ethanol (95) (1 in 5000), cool to 10 °C, and allow to stand for 30 minutes while vigorous shaking occasionally: A precipitate is produced.

Difenidol hydrochloride $C_{21}H_{27}NO{\cdot}HCl$ [Same as the namesake monograph]

1,3-Dihydroxynaphthalene $C_{10}H_6(OH)_2$ Purple-brown, crystals or powder. Freely soluble in water and in ethanol (95).

Melting point : about 125 °C

Diisopropylamine $[(CH_3)_2CH]_2NH$ Colorless, clear liquid, having an amine like odor. Miscible with water and with ethanol (95). The solution in water is alkaline.

Refractive index n_D^{20}: 1.391 ~ 1.394

Specific gravity d_{20}^{20}: 0.715 ~ 0.722

Diltiazem hydrochloride $C_{22}H_{26}N_2O_4S.HCl$ [Same as the namesake monograph]

Dilute acetic acid See acetic acid, dilute.

Dilute bismuth subnitrate-potassium iodide TS for spray Dissolve 10 g of tartaric acid in 50mL of water, and add 5mL of bismuth subnitrate TS.

Dilute bromophenol blue TS See bromophenol blue TS, dilute.

Diluted ethanol See ethanol, diluted.

Dilute ethanol See ethanol, dilute.

Dilute ferric ammonium sulfate TS See ammonium iron (Ill) sulfate TS, dilute.

Dilute ferric chloride TS See iron (Ill) chloride TS, dilute.

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 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 *p*-dimethylaminobenzaldehyde-ferric chloride TS See 4-dimethylaminobenzaldehyde-iron (III) chloride 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.

Dilate 4-dimethylaminobenzaldehyde-iron (III) chloride TS See 4-dimethylaminobenzaldehyde-iron (III) chloride, dilute.

Dimedon $C_8H_{12}O_2$ White to pale yellowe, crystalline powder.
Melting point 145-149 °C

***N,N*-Dimethylacetamide** $CH_3CON(CH_3)_2$ Clear and colorless liquid.
Boiling point 163 ~ 165 °C
Specific gravity d_{20}^{20} 0.938 ~ 0.945 (Method 3).
Water Not more than 0.2 % (0.1 g, coulometric titration).
Purity Perform the test with 3 mL of *N,N*-dimethylactamide as directed under Gas Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of *N,N*-dimethylacetamide by the area percentage method: not less than 98.0 %.

Operating conditions
Detector: A hydrogen flamc-ionization detector.
Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface 0.5 um in thickness with polyethylene glycol 20 M for gas chromatography.
Column temperature: A constant temperature of about 70 °C, keep this temperature for 1 minute, then raise to 200 °C at a rate of 10 °C per minute, and keep 200 °C for 3 minutes.
Carrier gas: Helium
Flow rate (linear velocity): About 30 cm/sec.
System suitability
Test for required detectability: To exactly 1.0 g of *N,N*-dimethylacetamide add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Confirm that the peak area of *N,N*-dimethyactamide obtained from 3 μL of this solution is equivalent to 40 % to 60 % of the full-scale.
System repeatability: When the test is repeated with 3 μL of *N,N*-dimethylacetamide under the above operating conditions, the relative standard deviation of the peak area of *N,N*-dimethylacetamide is not more than 2.0 %.
Time span of measurement: About 2 times as long as the retention time of *N,N*-dimethylacetamide.

Dimethylamine $(CH_3)_2NH$, Colorless, clear liquid, having amine-like, characteristic odor. It is miscible with water or ethanol (99.5). It is alkaline.
Specific gravity d_{20}^{20} between 0.85 and 0.93.
Content between 38.0 and 45.0 %.
Assay Weigh accurately about 1.0 g of dimethylamine, transfer to a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid VS, and titrate the excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.5 mol/L sulfuric acid VS
= 45.08 mg of $(CH_3)_2NH$

***p*-Dimethylaminobenzaldehyde** See 4-dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde $(CH_3)_2NC_6H_4CHO$ [p-Dimethylaminobenzaldehyde, Special class].

***p*-Dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS.

***p*-Dimethylaminobenzaldehyde-ferric chloride TS, dilute** See 4-dimethylaminobenzaldehyde-iron (III) chloride, 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, then add 0.05 mL of iron (III) chloride TS. Use within 7 days.

4-Dimethylaminobenzaldehyde-iron (III) chloride TS, dilute To 80 mL of water add carefully 100 mL of 4-dimethylamino-benzaldehyde-iron (III) chloride

TS and 0.15 mL of iron (III) chloride TS, while cooling with ice.

***p*-Dimethylaminobenzaldehyde TS** See 4-dimethylaminobenzaldehyde TS.

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.

***p*-Dimethylaminobenzaldehyde TS for spraying** See p-dimethylaminobenzaldehyde TS for spraying.

4-Dimethylaminobenzaldehyde TS for spraying Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20mL of dilute sulfuric acid. Prepare before use.

***p*-Dimethylaminobenzylidenerhodanine** See 4-dimethylaminobenzylidenerhodanine.

4-Dimethylaminobenzylidenerhodanine $C_{12}H_{12}N_2OS_2$ [Special class].

***p*-Dimethylaminobenzylidenerhodanine TS** See 4-dimethylaminobenzylidenerhodanine TS.

4-Dimethylaminobenzylidenerhodanine TS Dissolve 0.02 g of 4-dimethylaminobenzylidenerhodanine in acetone to make 100 mL.

***p*-Dimethylaminocinnamaldehyde** See 4-dimethylaminocinnamaldehyde.

4-Dimethylaminocinnamaldehyde $C_{11}H_{13}NO$, Orange crystals or crystalline powder, having a characteristic odor. Freely soluble in dilute hydrochloric acid, sparingly soluble in ethanol (95) or diethyl ether, and practically insoluble in water.
Melting point between140 °C and 142 °C
Purity Clarity of solution Dissolve 0.2 g of 4-dimethyl-aminocinnamaldehyde in 20 mL of ethanol (95): the solution is clear.
Loss on drying not more than 0.5 % (1 g, 105 °C, 2 hours).
Residue on ignition not more than 0.10 % (1 g).
Nitrogen content 7.8 - 8.1 % (105 °C, 2 hours, after drying, according to the Nitrogen Determination).

***p*-Dimethylaminocinnamaldehyde TS** See 4-dimethylaminocinnamaldehyde TS.

4-Dimethylaminocinnamaldehyde TS Before use, add 1mL of acetic acid (100) to 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 2000).

Dimethylaniline See N,N-dimethylaniline.

Dimethylformamide See N,N-dimethylformamide.

***N,N*-Dimethylformamide** $HCON(CH_3)_2$ [Special class].

Dimethylglyoxime $C_4H_8N_2O_2$ [Special class].

Dimethylglyoxime-thiosemicarbazide TS *Solution A*: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100mL. Prepare before use. *Solution B*: Dissolve 0. 1 g of thiosemicarbazide in 50 mL of water with the acid of 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, and allow the mixture to stand for 1 hour. Use within 24 hours.

Dimethylglyoxime TS Dissolve 1 g of dimethylglyoxime in ethanol (95) to make 100mL.

***N,N*-Dimethyl-*n*-octylamine** $C_{10}H_{23}N$ Colorless liquid.
Refractive index n_D^{20} : 1.424

Dimethyl phthalate $C_{16}H_{22}O_4$, Colorless, clearliquid, having a slight aroma.
Refractive index n_D^{20} between 1.491 and 1.493.
Purity To 6.0mL of a solution of Dimethyl phthalate in isooctane (1 in 100) add a solution of n-amyl alcohol in hexane (3 in 1000) to make 50mL, 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 principal peak does not appear.

***N,N*-Dimethyl-*p*-phenylenediamine dichloride** $H_8N_2C_6H_4N(CH_3)_2 . 2HCl$ [*N,N*-Dimethyl-*p*-phenylenediammonium dichloride, Special class].

Dimethylsulfoxide CH_3SOCH_3 [Special class].

***m*-Dinitrobenzen** See 1,3-dinitrobenzen.

1,3-Dinitrobenzene $C_6H_4(NO_2)_2$ Light yellow to reddish-yellow crystals or crystalline powder.
Melting point : 88 ~ 92 °C.
Preserve in a light-resistant tight container.

1,3-Dinitrobenzene TS Dissolve 1 g of 1,3-dinitrobenzene in 100 mL of ethanol (95). Prepare before use.

1,3-Dinitrobenzene TS, alkaline Mix 1 mL of tetramethylammonium hydroxide and 140 mL of ethanol (99.5), titrate a part of the mixture with 0.01 mol/L hydrochloric acid VS, and dilute the remainder with ethanol (99.5) to give a 0.008 mol/L solution. Before

use, mix 40 mL of this solution with 60 mL of a solution of 1,3-dinitrobenzene in benzene (1 in 20).

***m*-Dinitrobenzen TS** See 1,3-dinitrobenzen TS.

2,4-Dinitrochlorobenzene See l-chloro-2,4-dinitrobenzene.

2,4-Dinitrofluorobenzene See 1-fluoro-2,4-dinitrobenzene.

2,4-Dinitrofluorobenzene TS See 1-fluoro-2,4-dinitro-benzene TS.

***m*-Dinitrobenzen TS, alkaline** See 1,3-dinitrobenzen TS, alkaline.

2,4-Dinitrophenol $C_6H_4N_2O_6$ Yellow crystals or crystalline power.
Melting point : 110 ~ 114 °C

2,4-Dinitrophenol TS Dissolve 0.5 g of 2,4-dinitrophenol in 100mL of ethanol (95).

2,4-Dinitrophenylhydrazine $(NO_2)_2C_6H_3NHNH_2$ [Special class].

2,4-Dinitrophenylhydrazine-benzene TS Dissolve 0.1 g of 2,4-dinitrophenylhydrazine in an amount of a solution of trichloroacetic acid in benzene (1 in 20) to make 100 mL, and filter if necessary.

2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100mL of diethylene glycol dimethyl ether while heating, cool, and 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 10mL of water, then add a mixture of 1 volume of aldehyde-free ethanol and 3 volumes of water to make 100mL, and filter if necessary.

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, and filter, if necessary.

Dinonyl phthalate $C_6H_4(COOC_9H_{19})_2$, Colorless to pale yellow, clear liquid.
Specific gravity d_{20}^{20} between 0.967 and 0.987.
Acid value not more than 2.

Dioxane See 1,4-dioxane.

1,4-Dioxane $C_4H_8O_2$[Special class].

Diphenhydramine $C_{17}H_{21}NO$ [Same as the namesake monograph].

Diphenyl $C_{12}H_{10}$ White crystals or crystalline powder, having a characteristic odor. Freely soluble in acetone or diethyl ether, soluble in ethanol (95), and practically insoluble in water.
Melting point between 68 °C and 72 °C
Purity Dissolve 0.1 g of diphenyl in 5mL 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. Measure each peak area by the automatic integration method and calculate the amount of diphenyl by the area percentage method: it is not less than 98.0 %.

Operating conditions
Detector: Hydrogen flame-ionization detector.
Column: A glass tube, about 3 mm in inside diameter and about 2 m in length, packed with 10 % of polyethylene glycol 20 m for thin-layer chromatography supported on 150 to 180 μm mesh siliceous earth for gas chromatography.
Column temperature: A constant temperature of about 180 °C.
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 1.0mL of the test solution to make 100 mL, is 5 % to 15 % of the full scale.
Time span of measurement: About 3 times as long as the retention time of diphenyl after the solvent peak.

Diphenylamine $(C_6H_5)_2NH$ [Special class].

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 diphenilamine-acetic acid (100) TS.

Diphenylamine TS Dissolve 1 g of diphenylamine in 100mL of sulfuric acid. Use the colorless solution.

9,10-Diphenylanthracene $C_{26}H_{18}$ Yellow crystalline powder.
Soluble in diethyl ether, and practically insoluble in water.
Melting point : about 248 °C

Diphenylcarbazide See 1,5-diphenylcarbazide.

1,5-Diphenylcarbazide $C_{13}H_{14}N_4O$ [Special class].

Diphenylcarbazide TS See 1,5-diphenylcarbazide TS.

1,5-Diphenylcarbazide TS Dissolve 0.2 g of 1,5-diphenylcarbazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9:1).

Diphenylcarbazone $C_{13}H_{12}N_4O$ A yellowish red crystalline powder.
Identification. Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry : it exhibits absorption at the wave numbers of about 1708 cm^{-1}, 1602 cm^{-1}, 1497 cm^{-1}, 1124 cm^{-1}, 986 cm^{-1}, 748 cm^{-1} and 692 cm^{-1}.
Preserve in a light-resistant tight container.

Diphenylcarbazone TS Dissolve 1 g of diphenylcarba-zone in ethanol (95) to make 1000 mL.

Diphenyl ether $C_{12}H_{10}O$ Colorless crystals, having a geranium-like aroma. Dissolves in alcohol (95) or diethyl ether, and practically in soluble in water.
Specific gravity d_{20}^{20} between 1.072 and 1.075.
Boiling point between 254 °C and 259 °C
Melting point 28 °C

Diphenyl phthalate $C_6H_4(COOC_6H_5)_2$ White crystalline powder.
Melting point between 71 °C and 76 °C
Purity Related substances Dissolve 0.06 g of diphenyl phthalate in 50mL of chloroform, and use this solution as the test solution. Proceed with 10 μL of the test solution as directed in the Assay under Tolnaftate Solution: any peak other than the principal 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 as long as the retention time of diphenyl phthalate after the solvent peak.

Dipotassium hydrogen phosphate K_2HPO_4 [Special class].

Dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3 Mix 100mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and 38mL of 1 mol/L citric acid TS for buffer solution, and add water to make 200 mL.

1 mol/L Dipotassium hydrogen phosphate TS for buffer solution Dissolve 174.18 g of dipotassium hydrogen phosphate in water to make 1000 mL.

2,2´-Dipyridyl. $C_{10}H_8N_2$ [Special class]

***a,a´*-Dipyridyl** See 2,2´-dipyridyl.

Disodium hydrogen phosphate See Disoduim hydrogen phosphate 12-hydrate

Disodium hydrogen phosphate-citric add buffer solution, pH 4.5 Dissolve 21.02 g of citric acid monohydrate in water to make 1000mL, and adjust the pH to 4.5 with a solution prepared by dissolving 35.82 g of disodium hydrogen phophate 12-hydrate in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 Dissolve 28.4 g of 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 becomes 6.0 (ratio of volume: About 63 : 37).

Disodium hydrogen phosphate for pH determination Na2HPO4 [for pH determination]

Disodium hydrogen phosphate TS Dissolve 12 g of disodium hydrogen phosphate 12-hydrate in water to make 100 mL (1/3 mol/L).

0.05 mol/L Disodium hydrogen phosphate TS Dissolve 7.0982 g of disodium hydrogen phosphate in water to make 1000 mL.

0.5 mol/L Disodium hydrogen phosphate TS Dissolve 70.982 g of disodium hydrogen phosphate in water to make 1000 mL.

Disoduim hydrogen phosphate 12-hydrate $Na_2HPO_4 . 12H_2O$ [Special class].

Dithiothreitol $C_4H_{10}O_2S_2$ Crystals.
Melting point : about 42 °C

Dithizone $C_6H_5NHNHCSN$ [Special class].

Dithizone solution for extraction Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of ethanol (95). Store in a cold place. Before use, shake a suitable volume of the solution with one-half of its volume of diluted nitric acid (1 in 100), and use the chloroform layer after discarding the water layer.

Dithizone TS Dissolve 25 mg of dithizone in ethanol (95) to make 100mL. Prepare before use.

1,3-Di (4-pyridyl) propane $C_{13}H_{14}N_2$ A pale yellow powder.
Melting point between 61 °C and 62 °C
Water Less than 0.1 %

1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-l-diprotine $C_{18}H_{28}N_2O_6S_2$ White, crystals 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-diprotine according to potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 2960 cm^{-1}, 1750 cm^{-1},1720 cm^{-1},1660 cm^{-1},1480 cm^{-1},1450 cm^{-1} and1185 cm^{-1}.

Purity Related substances—Disslove about 0.10 g of 1,1´-[3,3´-Dithiobis(2-methyl-1-oxopropyl)]-l-diprotine 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 a plate of silica gel for thin-layer chromatography. 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 observable spot other than the principal spot (R_f value: about 0.2) appears.

Content not less than 99.0 %

Assay Weigh accurately about 0.3 g of 1,1´-[3,3´-Dithiobis(2-methyl-1-oxopropyl)]-l-diprotine, dissolve in 20 mL of methanol, add 50mL of water, and titirate with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through bluish green to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank dtermination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide VS
= 21.628 g of $C_{18}H_{28}N_2O_6S_2$

Dragendorff's TS Dissolve 0.85 g of bismuth subnitrate in 10mL of acetic acid (100) and 40mL of water with vigorous shaking (solution A). Dissolve 8 g of potassium iodide in 20mL of water (solution B). Immediately before use, mix equal volumes of solutions A and B and acetic acid (100). Store solutions A and B in light-resistant containers.

Dragendorff's TS for spraying Add 20mL of diluted acetic acid (31) (1 in 5) to 4mL of a mixture of equal volumes of solutions A and B of Dragendorff's TS. Prepare before use.

EMB plate medium Melt eosin methylene blue agar medium by heating, and cool to about 50 °C. Transfer about 20mL of this 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.

Enzyme TS The supernatant liquid is obtained as follows: To 0.3 g of an enzyme preparation potent in amylolytic and phosphorolytic activities, obtained from Aspergillus oryzae, 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 See Eosin Y.

Eosin methylene blue agar medium Dissolve by boiling 10 g of casein peptone, 2 g of dipotassium hydrogen-phosphate and 25 g to 30 g of agar in about 900 mL of water. To this mixture, add 10 g of lactose monohydrate, 20 mL of a solution of eosin Y (1 in 50), 13mL of a solution of methylene blue (1 in 200), and warm water to make 1000 mL. Mix thoroughly, dispense, sterilize by autoclaving at 121 °C for not more than 20 minutes, and cool quickly by immersing in cold water, or sterilize fractionally on each of three successive days for 30 minutes at 100 °C.

Eriochrome black T $C_{20}H_{12}N_3NaO_7S$ [Specialclass].

Eriochrome black T-sodium chloride indicator Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate until the mixture becomes homogeneous.

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. Preserve in light-resistant containers.

Erythritol $C_4H_{10}O_4$ [Special class].

Erythromycin C $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

Ethanol See ethanol (95).

Ethanol, aldehyde-free Transfer 1 L 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. In a separate container, 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 supernatant liquid, and distil the ethanol.

Ethanol, dehydrate See ethanol (99.5).

Ethanol, dilute To 1 volume of ethanol (95), add 1 volume of water. It contains 47.45 to 50.00 vol % of C_2H_5OH.

Ethanol, diluted Prepare by dluting ethanol (99.5).

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 VS until a light red color develops. Prepare before use.

Ethanol (95) C_2H_5OH [Special class].

Ethanol (99.5) C_2H_5OH [Special class].

Ethenzamide $C_9H_{11}NO_2$ [Same as the namesake monograph].

Ether $C_2H_5OC_2H_5$ [Special class].

Ether, anesthetic $C_2H_5OC_2H_5$ [Same as the namesake monograph].

Ether, anhydrous $C_2H_5OC_2H_5$ [Special class. The water content is not more than 0.01 %]

Ethinylestradiol $C_{20}H_{24}O_2$ [Same as the namesake monograph]

***p*-Ethoxyphenol** See 4-ethoxyphenol.

4-Ethoxyphenol $C_8H_{10}O_2$, White to light yellow-brown crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.
Melting point between 62 °C and 68 °C
Purity Dissolve 0.5 g of 4-Ethoxyphenol in 5mL of ethanol (95), and use this solution as the test solution. Perform the test 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 substance other than 4-ethoxyphenol by the area percentage method: it is not more than 2.0 %.

Operating conditions
Detector: Thermal conductivity detector.
Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with mothylsilicone polymer for gas chromatography supported on 180- to 250-μM siliceous earth for gas chromatography.
Column temperature: A constant temperature of about 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 detection sensitivity so that the peak height of 4-ethoxyphenol obtained from 1 μL of the test solution is not less than 50 % of the full scale.
Time span of measurement: 3 times as long as the retention time of 4-ethoxyphenol after the peak of solvent.

Ethyl acetate $CH_3COOC_2H_5$ [Special class].

Ethyl cyanoacetate $NCCH_2COOC_2H_5$ Colorless or light yellow, clear liquid, having an aromatic odor.
Specific gravity d_{20}^{20} about 1.08
Identification To 0.5 mL of a solution in ethanol (99.5) (1 in 10,000) add a mixture of 1mL of a solution of quinhydrone in diluted ethanol (99.5) (1 in 2) (1 in 20,000) and 1 drop of ammonia solution (28): a light blue color develops.

Ethylenediamine $C_2H_8N_2$ [Same as the namesake monograph in Part II].

Ethylenediamine TS Dissolve 70 g of ethylenediamine in 30 g of water.

Ethylene glycol $HOCH_2CH_2OH$ [Special class].

Ethylene glycol for Karl Fischer method Distil ethyleneglycol, and collect the fraction distilling between 195 °C and 198 °C. The water content is not more than 1.0 mg permL.

Ethyl formate $C_3H_6O_2$ [Special class]

Ethyl iodide C_2H_5 [Special class]

***N*-Ethylmaleimide** $C_6H_7NO_2$ White crystals, having a pungent, characteristic odor. Freely soluble in ethanol (95), and slightly soluble in water.
Melting point between 43 °C and 46 °C
Purity Clarity and color of solution Dissolve 1 g of *N*-ethylmaleimide in 20 mL of ethanol (95): the solution is clear and colorless.
Content not less than 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 VS, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide VS
= 12.513 mg of $C_6H_7NO_2$

Ethyl parahydroxybenzoate $HOC_6H_4COOC_2H_5$ [Same as the namesake monographin Part II, Ethyl paraben]

Ethyl propionate $CH_3CH_2COOC_2H_5$ Colorless, clear liquid.
Specific gravity d_{20}^{20} between 0.890 and 0.892

Fehling's TS *The 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.
The alkaline tartrate solution Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make 500 mL. Preserve this solution in polyethylene containers.
Before use, mix equal volumes of both solutions.

Fehling's TS for amylolytic activity test *The copper solution* Dissolve 34.660 g of copper (II) sulfate pentahydrate, accurately weighed, in water to make exactly 500 mL. Preserve this solution in well-filled, glass-stoppered bottles.
The alkaline tartrate solution Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodiumhydroxide in water to make exactly 500mL. Preserve this solution in polyethylene containers.

Before use, mix exactly equal volumes of both solutions.

Ferric ammonium citrate See ammonium iron (III) citrate.

Ferric ammonium sulfate TS See ammonium iron (III) sulfate TS.

Ferric ammonium sulfate TS, dilute See ammonium iron (III) sulfate TS, dilute.

Ferric chloride See iron (III) chloride hexahydrate.

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 TS See iron (III) chloride TS.

Ferric chloride TS, dilute See iron (III) chloride TS, dilute.

Ferric nitrate See iron (III) nitrate enneahydrate.

Ferric nitrate TS See iron (III) nitrate TS.

Ferric perchlorate See iron (III) perchlorate hexahydrate.

Ferric perchlorate-dehydrated ethanol TS See iron (III) perchlorate-ethanol (99.5) TS.

Ferric salicylate TS Dissolve 0.1 g of ammonium iron (III) sulfate 12-hydrate in 50 mL of diluted sulfuric acid (1 in 250), and add water to make 100 mL. Measure 20 mL of this solution, and add 10 mL of a solution of sodium salicylate (23 in 2000), 4 mL of dilute acetic acid, 16 mL of sodium acetate TS, and water to make 100 mL. Prepare before use.

Ferric sulfate See iron (III) sulfate n-hydrate.

Ferric sulfate TS See iron (III) sulfate TS.

Ferroin TS Dissolve 0.7 g of iron(II) sulfate heptahydrate and 1.76g of o-phenanthrolinemonohydrochloride

Ferrous ammonium sulfate See ammonium iron (II) sulfate hexahydrate.

Ferrous sulfide See iron (II) sulfide.

Ferrous tartrate TS See iron (II) tartrate TS.

Fibrinogen Fibrinogen is prepared from human or bovine blood by fractional precipitation with ethanol or ammonium sulfate. It may contain citrate, oxalate, and sodium chloride. A white, amorphous solid. Add 1mL of isotonic sodium chloride solution to 0.01 g of fibrinogen. It, when warmed to 37 °C, dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin.

Fluocinolone acetonide $C_{24}H_{30}F_2O6$ [Same as the namesake monograph].

Fluorescein $C_{20}H_{12}O_5$ [Special class].

Fluorescein sodium $C_{20}H_{10}Na_2O_5$ [Same as the namesake monograph].

Fluorescein sodium TS Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

1-Fluoro-2,4-Dinitrobenzene $C_6H_3(NO_2)_2F$ Light yellow liquid or crystalline masses.
Melting point : about 25 °C.
Identification. Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry : it exhibits absorption at the wave numbers of about 3110 cm^{-1}, 1617 cm^{-1}, 1538 cm^{-1}, 1345 cm^{-1}, 1262 cm^{-1} and 743 cm^{-1}.
Preserve in a light-resistant tight container.

1-Fluoro-2,4-Dinitrobenzene TS Dissolve 1.0 g of 1-Fluoro-2,4-Dinitrobenzene in ethanol to make 100mL.

Folic acid $C_{19}H_{19}N_7O_6$ [Same as the namesake monograph].

Folin's 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 monohydrate and 10mL 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, and cool. Add water to make 200mL, 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-sulfuric acid TS Add 1 drop of formaldehyde solution to 1mL of sulfuric acid. Prepare before use.

Formaldehyde solution TS To 0.5mL of formaldehyde solution, add water to make 100mL.

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 method $HCONH_2$ [Special class; water content per g of formamide for Karl Fischer method should be not more than 1 mg].

Formic acid HCOOH [Special class, specific gravity: not less than 1.21].

Freund's complete adjuvant A suspension of 5 mg of mycobacteria of Corynebacterium butyricum, killed by heating, in 10 mL of a mixture of mineral oil and aricel A (17:3).

Fructose $C_6H_{12}O_6$ [Same as the monograph Fructose].

Fuchsin A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95).
Loss on drying between 17.5 and 20.0 % (1 g, 105 °C, 4 hours)
Residue on ignition not more than 0.1 % (1 g).

Fuchsin-ethanol TS Dissolve 11 g of fuchsin in 100mL 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, and 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].

Gauze [Same as the namesake monograph in Part II].

D-Galactosamine hydrochloride $C_6H_{13}NO_5 \cdot HCl$
White powder.
Melting point : about 180 °C (with decomposition).

Gelatin [Same as the namesake monograph in Part II].

Gelatin, acid-treated [Same as the monograph in Part II Gelatin. Its isoelectric point is at pH between 7.0 and 9.0].

Gelatin peptone See peptone, gelatin.

Gelatin-phosphate buffer solution Dissolve 13.6 g of monobasic potassium phosphate, 15.6 g of monobasic sodium phosphate, 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, after cooling, 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 dehydrate, 5.96 g of disodium hydrogen phosphate 12-hydrate and 54.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g gelatin to this solution by heating, and after cooling add water to make 600 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, mix these solutions, and 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 dissolve by heating, adjust to pH 8.0 with dilute hydrochloric acid after cooling, and add water to make 600 mL.

Gelatin TS Dissolve 1 g of gelatin in 50 mL of water by gentle heating, and filter, if necessary. Prepare before use.

Giemsa's TS Dissolve 3 g of azure II-eosin and 0.8 g of azure II in 250 g of glycerin by warming to 60 °C. After cooling, add 250 g of methanol, and mix well. Allow to stand for 24 hours, and filter. Store in tightly stoppered bottles. Azure II-eosin is prepared by coupling eosin to azure II. Azure II is the mixture of equal quantities 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 crystals or crystalline powder.
Content : not less than 98 %.
Assay. Dissolve about 0.4 g of D-glucosamine hydrochloride, accurately weighed, in 50mL of water, add 5

mL of diluted nitric acid(1in3), 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 $C_6H_{12}O_6$ [Same as the namesake monograph].

Glucose-pepton medium See the Sterility Test under the General Tests, Processes and Apparatus.

Glucose TS Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

D-glucuronic acid $C_6H_{10}O_7$ [Special class].

L-Glutamic acid $HOOC(CH_2)_2CH(NH_2)COOH$ [Special class].

7-(Glutarylglycyl-L-arginylamino)-4-methylcoumarin TS Dissolve 5 mg of 7-(glutarylglycyl-L-arginyl-amino)-4-methyl-coumarin in 0.5 to 1 mL of acetic acid (100), lyophilize, dissolve this in 1mL of dimethylsulfoxide, and use this solution as solution-A. Dissolve 30.0 g of trishydroxymethylaminomethane and 14.6 g of sodium chloride in 400 mL of water, adjust the pH to 8.5 with dilute hydrochloric acid, add water to make 500 mL, and use this solution as solution-B. Mix 1mL of the solution-A and 500 mL of the solution-B before use.

Glycerin $C_3H_8O_3$ [Glycerol, Specialclass. Same as the monograph Concentrated Glycerin].

Glycine H_2NCH_2COOH [Special class].

Griess-Romijin's nitric acid reagent Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid, and 1.5 g of zinc dust in a mortar.
Storage Preserve in tight, light-resistant containers.

Griess-Romijin's nitrous acid reagent Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid, and 89 g of tartaric acid in a mortar.
Storage Preserve in tight, light-resistant containers.

Guaiacol $CH_3OC_6H_4OH$, Clear, colorless to yellow liquid or colorless crystals, having a characteristic aroma. Sparingly soluble in water, and miscible with ethanol (95), with diethyl ether and with chloroform.
Melting point : About 28 °C
Purity Perform the test with 0.5 μL of guaiacol 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 guaiacol by the area percentage method: not less than 99.0 %.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μm in particle diameter, coated with polyethylene glycol 20 m at the ratio of 20 %.

Column temperature: A constant temperature of about 200 °C.

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 of guaiacol obtained from 0.5 μL of guaiacol is about 90 % of the full scale.
Time span of measurement: About 3 times as long as the retention time of guaiacol.

Helium He not less than 99.995 vol %.

Hematoxylin $C_{16}H_{14}O_6 \cdot nH_2O$ White or light yellow to brownish crystals or crystalline powder. It is soluble in hot water and in ethanol (95), and sparingly soluble in cold water.
Residue on ignition not more than 0.1 % (1 g).

Hematoxylin TS Dissolve 1 g of hematoxylin in 12mL of dehydrated ethanol (95). Dissolve 20 g of aluminum potassium sulfate 12-hydrate 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 [Same as the namesake monograph].

Heptane $CH_3(CH_2)_5CH_3$ [Special class].

Hexamethylenetetramine $(CH_2)_6N_4$ [Special class].

Hexamine See hexamethylenetetramine.

Hexane C_6H_{14}[Special class].

Hexane for liquid chromatography $CH_3(CH_2)_4CH_3$ Colorless, clear liquid. Miscible with ethanol (95), diethyl ether, chloroform or benzene.
Boiling point About 69 °C
Purity (1) *Ultraviolet absorptive substances* Read the absorbances of n-hexane for liquid chromatography as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: not more than 0.3 at the wavelength of 210 nm, and not more than 0.01 between 250 nm and 400 nm. (2) *Peroxide* To a mixture of 100mL of water and 25 mL of dilute sulfuric acid add 25mL of a solution of potassium iodide (1 in 10) and 20 g of n-hexane for thin-layer chromatography. Stopper tightly, shake, and allow to stand in a dark place for 15 minutes. Titrate this solution, while shaking well, with 0.01 mol/L sodium thiosulfate (indicator:

lmL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

n-Hexane for liquid chromatography See hexane for liquid chromatography.

Hexane for spectrophotometry [Special class]. When determining the absorbance as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank solution, its value is more than 0.10 at 220 nm and more than 0.02 at 260 nm, and it has no characteristic absorption between 260 nm and 350 nm.

n-Hexane for spectrophotometry See hexane for spectrophotometry.

2-Ethylhexanoic acid $C_8H_{16}O_2$ Colourless liquid
Purity Related substances Dissolve 0.2 g of 2-ethylhexanoic acid in 5mL of water, add 3mL of dilute hydrochloric acid and 5mL of hexane, shake of 1 min, allow th layers to separate and use the upper layer. Perform the test with 1 μL of the test solution as directed under the gas Chromatography according to the component determination under Amoxicillin sodium: the total area of the peaks other than the principal peak, apart from the principal peak and the peak due to the solvent, is not greater than 2.5 % of the principal peak.

L-Histidine hydrochloride See L-histidine hydrochloride monohydrate.

L-Histidine hydrochloride monohydrate $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ [Special class].

Honokiol $C_{16}H_{18}O_2 \cdot xH_2O$ 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 the test solution as directed in the Component determination under Magnolia Bark: total area of peaks other than honokiol from the test solution is not larger than 1/10 of total area of the peaks other than the solvent peak.

Horse serum Collect the blood from horse in a flask, coagulate, and allow to stand at room temperature until the serum is separated. Transfer the separated serum in glass containers, and preserve at -20 °C.

Hydralazine hydrochloride $C_8H_8N_4.HCl$ [Same as the namesake monograph].

Hydrazine dihydrochloride $(NH_2)_2 \cdot 2HCl$ White powder
Assay Dissolve about 34 mg, accurately weighed, in 50 mL of water. Add carefully while stirring, 1g of sodium bicarbonate.Titrate with 0.1 N iodine solution, determining the endpoint potentiometrically.

EachmL of 0.1 mol/L Iodine VS
= 2.36 mg of $(NH_2)_2 \cdot 2HCl$

Hydrazine monohydrate $NH_2NH_2.H2O$ Colorless liquid, having a characteristic odor.

Hydrazine sulfate See hydrazinum sulfate.

Hydrazinum sulfate $N_4H_6SO_4$[Special class].

Hydrazinum sulfate TS Dissolve 1.0 g of hydrazinum sulfate in water to make 100mL.

Hydrobromic acid HBr [Special class].

Hydrochloric acid HC1 [Special class].

Hydrochloric acid, dilute Dilute 23.6 mL of hydrochloric acid with water to make 100 mL (10 %).

Hydrochloric acid-ethanol TS See hydrochloric acid-ethanol (95) TS.

Hydrochloric acid-ethanol (95) TS Dilute 23.6 mL of hydrochloric acid with ethanol (95) to make 100 mL.

Hydrochloric acid-potassium chloride buffer solution, pH 2.0 To 10.0mL of 0.2 mol/L hydrochloric acid VS, add 88.0 mL of 0.2 mol/L potassium chloride TS, adjust the pH to 2.0 ± 0.1 by, adding 0.2 mol/L hydrochloric acid VS, then, add water to make 200 mL.

Hydrochloric acid, purified Add 0.3 g of potassium permanganate to 1000 mL of diluted hydrochloric acid (1 in 2), distil, discard the first 250 mL of the distillate, and collect the following 500 mL of the distillate.

0.001 mol/L Hydrochloric acid TS Dilute 10 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.01 mol/L Hydrochloric acid TS Dilute 100 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.02 mol/L Hydrochloric acid TS Dilute 100 mL of 0.2 mol/L hydrochloric acid TS with water to make 1000 mL.

0.05 mol/L Hydrochloric acid TS Dilute 100 mL of 0.5 mol/L hydrochloric acid TS with water to make 1000 mL.

0.1 mol/L Hydrochloric acid TS Dilute 100 mL of 1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.2 mol/L Hydrochloric acid TS Dilute 18 mL of hydrochloric acid with water to make 1000 mL.

0.5 mol/L Hydrochloric acid TS Dilute 45 mL of hydrochloric acid with water to make 1000 mL.

1 mol/L Hydrochloric acid TS Dilute 90 mL of hydrochloric acid with water to make 1000 mL.

2 mol/L Hydrochloric acid TS Dilute 180 mL of hydrochloric acid with water to make 1000 mL.

3 mol/L Hydrochloric acid TS Dilute 270 mL of hydrochloric acid with water to make 1000 mL

5 mol/L Hydrochloric acid TS Dilute 450 mL of hydrochloric acid with water to make 1000 mL.

6 mol/L Hydrochloric acid TS Dilute 540 mL of hydrochloric acid with water to make 1000 mL.

7.5 mol/L Hydrochloric acid TS Dilute 675 mL of hydrochloric acid with water to make 1000 mL.

0.1 mol/L Hydrochloric acid TS for bacterial endotoxins test To 9.0 mL of hydrochloric acid, add water for bacterial endotoxins test to make 1000 mL.

Hydrocortisone $C_{21}H_{32}O_6$ [Same as the namesake monograph].

Hydrocortisone acetate $C_{23}H_{32}O_6$ [Same as the namesake monograph].

Hydrofluoric acid HF [Special class]. It contains not less than 46.0 % of HF.

Hydrogen H_2 [Standard substance, Third class]. It contains not less than 99.99 % of H2.

Hydrogen chloride-ethanol TS See hydrogen chloride-ethanol (99.5) TS.

Hydrogen chloride-ethanol (99.5) TS Pass dry hydrogen chloride, which is generated by slowly, adding 100 mL of sulfuric acid drop-wise to 100 mL of hydrochloric acid, and dried by washing with sulfuric acid, through 75 g of ethanol (99.5) cooled in an ice-bath until the increase in mass has reached 25 g. 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 a purple-blue color develops. Prepare before use.

Hydrogen peroxide TS Dilute 1 volume of hydrogen peroxide (30) with 9 volumes of water. Prepare before use (3 %).

Hydrogen peroxide TS, dilute Dilute 1mL of hydrogen peroxide (30) with 500mL of water, and dilute 5mL of this solution with water to make 100mL. Prepare before use.

Hydrogen peroxide water, strong See hydrogen peroxide (30).

Hydrogen peroxide (30) H_2O_2 [Hydrogen peroxide, Special class, Concentration: between 30.0 and 35.5 %].

Hydrogen sulfide H_2S, Acolorless, poisonous gas, heavier than air. It dissolves in water. Prepare by treating iron (II) sulfide heptahydrate with dilute sulfuric acid or dilute hydrochloric acid. Other sulfides yielding hydrogen sulfide with dilute acids may be used.

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.

Hydroquinone $C_6H_4(OH)_2$ [Special class].

***m*-Hydroxyacetophenone** $C_8H_8O_2$ White to pale yellow crystals or 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, pH 4.5 (1 in 15000) as directed in the Assay under Cefalexin: Any obstructive peaks for determination of Cefalexin are not observed.

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl-2-(p-methoxyphenyl)-1,5-benzothiazepine-4(5H)-onehydrochloride** See *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl].-2-(4-hoxyphenyl)-1,5-benzothiazepine-4-(5H)-onehydrochloride.

***p*-Hydroxyacetophenone** $C_8H_8O_2$, White to pale yellow crystals or crystalline powder. It is freely soluble in methanol.
Melting point between107and111 °C
Purity Weigh 1 mg of p-hydroxyacetophenone, add methanol and dissolve to make exactly 10mL, and use this solution as the test solution. Perform the test with 20 μL of the test solution as directed under the Liquid Chromatography according to the component determination under Peony Root: the total area of the peaks other than the peak of *p*-hydroxyacetophenone from the test solution is not larger than 3/100 of the total area of the peak s other than the solvent peak.

2-Hydroxybenzyl Alcohol $C_7H_8O_2$ Off-white flakes. Very soluble in ethanol, in chloroform, and in ether; soluble in water and in benzene.
Assay Inject an appropriate specimen into a gas chromatograph, The area of the 2-Hydroxybenzyl Alcohol peak is not less than 99 % of the total peak area.

Operating conditions

Detector: A flame-ionization detector.

Column: A column, about 0.25 mm in inside diameter and about 30 m in length, packed with siliceous earth for gas chromatography, 1 μm in particle diameter.

Iinjection port temperature : about 250 °C

Column temperature: A column temperature is maintained at 150 °C and programmed to rise 10 °C per minute

Detector temperature: abort 300 °C

Carrier gas: Helium.

Melting point between 83 and 85 °C

_d_-3-Hydroxy-_cis_-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride $C_{20}H_{24}N_2O_3S{\cdot}HC1$, To 9 g of diltiazem hydrochloride, add 50 mL of ethanol (99.5), and dissolve by heating at 80 °C. To this solution, add slowly 50 mL of a solution of potassium hydroxide in ethanol (99.5) (33 in 500) drop-wise, and heat for 4 hours with stirring. Cool in an ice-bath, filter, and evaporate the filtrate to dryness. Dissolve the residue in ethanol (99.5), add slowly a solution of hydrochloric acid in ethanol (99.5) (59 in 250) to make acidic, and filter. Add diethyl ether slowly to the filtrate, and filter the crystals produced. To the crystals, add ethanol (99.5), heat to dissolve, add 0.5 g of activated charcoal, allow to stand and filter. After cooling the filtrate in an ice-methanol bath, filter the crystals formed, and wash with diethyl ether. Further, add ethanol (99.5) to the crystals, and heat to dissolve. After cooling, filter the crystals produced, and dry under reduced pressure. White crystals or crystalline powder, having a slight, characteristic odor.

Purity Dissolve 0.050 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepine-4-(5H)-one hydrochloride in chloroform to make exactly 10mL, and use this solution as the test solution. Perform the test with the test solution as directed under the Thin-layer Chromatography. Spot 20 μL of the test solution on a plate 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 : 1) to a distance of about 13 cm, and air-dry the plate. Spray evenly iodine TS on the plate: any spot other than the principal spot does not appear.

Water more than 1.0 % (0.5 g).

Content not less than 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-(p-methoxyphenyl)-1,5-benzothiazepine-4-(5H)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometrictitration). Perform a blank determination in the same manner, and make any necessary correction

EachmL of 0.1 mol/L perchloric acid VS
= 40.89 mg of $C_{20}H_{24}N_2O_3S{\cdot}HC1$

Hydroxylamine hydrochloride See hydroxylammonium chloride.

Hydroxylamine hydrochloride TS See hydroxylammonium chloride TS.

Hydroxylamine perchlorate $NH_2OH{\cdot}HClO_4$, Hygroscopic, white crystals. Dissolves in water or ethanol (95). Melting point between 87.5 and 90 °C

Hydroxylamine perchlorate-dehydrated ethanol TS See hydroxylamine perchlorate-ethanol (99.5) TS.

Hydroxylamine perchlorate-ethanol (99.5) TS Dilute 2.99 mL of hydroxylamine perchlorate TS with ethanol (99.5) to make 100 mL.

Storage Preserve in tight containers, in a cold place.

Hydroxylamine perchlorate TS Ethanol (95) solution which contains 13.4 % of hydroxylamine perchlorate.

Storage 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, with stirring, 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and filter. Prepare before use.

Hydroxylamine TS, alcoholic Dissolve 3.5 g of hydroxylamine hydrochloride in 95mL of ethanol (60 % v/v) add 0.5 mL of a 2 g/L solution of methyl orange in ethanol (60 % v/v) and sufficient 0.5 mol/L potassium hydroxide in ethanol (60 % v/v) to give a pure yellow colour. Dilute to 100 mL with ethanol (60 % v/v).

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 hydrochloride TS, pH 3.1 hydroxylammonium chloride TS, pH 3.1.

Hydroxylammonium chloride $NH_2OH.HCl$ [Special class].

Hydroxylammonium chloride-iron (III) chloride TS Acidify 100mL of a solution of iron (III) chloride hexahydrate in ethanol (95) (1 in 200) with hydrochloric acid, and dissolve 1 g of hydroxylammonium chloride in the solution.

Hydroxylammonium chloride TS Dissolve 20 g of hydroxylammonium chloride in water to make 65mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia solution (28) until the solu-

tion exhibits a yellow color. Shake well after adding 10mL of a solution of sodium N,N-diethyldithiocarbamate trihydrate (1 in 25), allow to stand for 5 minutes, and extract this solution with 10 to 15 mL volumes of chloroform. Repeat the extraction until 5 mL of the extract does not exhibit a yellow color, upon adding 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) and shaking it. Add 1 to 2 drops of thymol blue TS, add drop-wisedilute hydrochloric acid to this aqueous solution until it exhibits a red color, then add water to make 100mL.

Hydroxylammonium chloride TS, pH 3.1 Dissolve 6.9 g of hydroxylammonium chloride in 80mL of water, adjust the pH to 3.1 by adding dilute sodium hydroxide TS, and add water to make 100mL.

***N*-2-Hydroxyethylpiperazine-N'-2-ethanesulfonicacid** $C_8H_{18}N_2O_4S$ White crystalline powder.
Purity Clarity and color of solution. Dissolve 11.9 g of *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonicacid in 50 mL of water: the solution is clear and colorless.
Content : not less than 99.0 %.
Assay. Weigh accurately about 1 g of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonicacid, dissolve in 60 mL of water, and titrate with 0.5 mol/L sodium hydroxide VS (Potentiometrictitration).

EachmL of 0.5 mol/L sodium hydroxide VS
= 119.2 mg of $C_8H_{18}N_2O_4S$

1-(2-Hydroxyethyl)-1H-tetrazol-5-thiol $C_3H_6N_4OS$ White, crystals or powder.
Melting point between 136 and 141 °C
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 sample solution. Pipet 0.5 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot 1 mL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, 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 under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Hydroxy Naphthol Blue $C_{20}H_{14}N_2O_{11}S_3$ 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 existance of calcium ion, and a deep blue color with existance of disodium dihydrogen ethylendiamine tetraacetate.
Sensitivity for Quantiative Test of Calcium Dissolve 0.3 g of Hydroxy Naphthol Blue in 100mL of water, add 10mL of sodium hydroxide TS, add 1mL of a solution of calcium chloridecalcium 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.

***N*-(3-Hydroxyphenyl)acetamide** $C_8H_6NO_2$ White to pale yellowish white crystals. It is freely soluble in ethanol (95), and sparingly soluble in water.
Melting point 146-149 °C
Purity (1) *Clarityandcolorofsolution* Dissolve 0.1 g ofN-(3-Hydroxyphenyl) acetamide in 1000 mL of water: the solution is clear and colorless. (2) *Relatedsubstances* Dissolve 0.1g of *N*-(3-hydroxyphenyl)acetamide in 1000 mL of water. Pipet 10 mL of this solution, add 6.5 mL of acetonitirle and water to make exactly 50mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Aspoxicillin: any peak other than those of of *N*-(3-Hydroxyphenyl)a cetamide and the solvent does not apppear.

3-(p-Hydroxyphenyl)propionicacid $C_9H_{10}O_3$ White to light yellow-brown crystals or crystalline powder, having a faint, characteristic odor.
Content not less than 99.0 %.
Assay Weigh accurately about 0.2 g of 3-(p-hydroxyphenyl)propionicacid, previously dried (in vacuum 60 °C, 4 hours), dissolve in 5mL of methanol, add 45mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromothymol blue TS).

EachmL of 0.1 mol/L sodium hydroxide VS
= 16.617 mg of $C_9H_{10}O_3$

2-Hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid $C_{21}H_{14}N_2O_7S$ [2-Hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoicAcid, Special class].

Phosphinic acid H_3PO_2 Colorless or pale yellow viscous liquid.
Identification. (1) To 0.5 mL of phosphinic acid add 0.5 mL of hydrogen peroxide (30) and 0.5 mL of diluted sulfuric acid (1 in 6), and evaporate to nearly dryness on a water bath. After cooling, add 10 mL of water and 5 mL of ammonia TS, and add 5 mL of magnesia TS: a white precipitate is produced.
(2) To 1mL of phosphinic acid add the mixture of iodine TS (1 mL) and water (20 mL): the iodine color disappears.
Content : between 30.0 and 32.0 %.

Assay. Weigh accurately about 1.5 g of phosphinic acid, and dissolve in water to make exactly 250 mL. Pipet 25 mL of this solution into an iodine bottle, add exactly 50 mL of 0.05 mol/L bromine VS, 100 mL of water and 10 mL of diluted sulfuric acid (1 in 6), immediately stoppered, gently shake, and allow to stand for 3 hours. Then add 20 mL of potassium iodide TS, stopper immediately, shake vigorously, and titrate liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1mL of starch TS). Perform a blank determination in the same manner.

EachmL of 0.05 mol/L bromine VS
= 1.650 mg of H_3PO_2

Hypoxanthine $C_5H_4N_4$ White crystals or crystalline powder. Soluable in ammonia TS, sparingly soluble in dilute hydrochloric acid or hot water, very slightly soluble in water, and practically insoluble in methanol.
Purity Related substances Dissolve 5.0 mg of hypoxanthine in 100 mL of a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL. Proceed with this solution as directed in the Purity (4) under Mercaptopurine: any spot other than the principal spot at the R_f value of about 0.2 does not appear.
Content not less than 97.0 % and not more than 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 10mL of this solution, and dilute with phosphate buffer solution, pH 7.0, to make exactly 250 mL. Read the absorbance A of this solution at the wavelength of 250 nm as directed under the Ultraviolet-visible Spectrophotometry, using phosphate burr solution, pH7.0, as the blank solution.

Amount (mg) of $C_5H_4N_4$4O=A/779 × 250,000

Ibuprofen $C_{13}H_{18}O_2$[Same as the namesake monograph].

Imidazole $C_3H_4N_2$ White crystalline powder. Very soluble in water and in methanol.
Melting point between 89 and 92 °C
Absorbance $E_{1\,cm}^{1\%}$ (313 nm) not more than 0.031 (8 g, water, 100mL)

Imidazole TS Dissolve 8.25 g of Imidazole in 65mL of water, adjust the pH to 6.80 with 5 mol/L hydrochloric acid TS, and add water to make exactly 100mL.

Imidazole for Karl Fischer method See the Water Determination under the General Tests, Processes and Apparatus.

Iminodibenzyl $C_{14}H_{13}N$ White to light brown crystals or crystalline powder, having a slight, characteristic odor.
Melting point between 104 and 110 °C
Purity (1) *Clarity of solution* Dissolve 1.0 g of iminodibenzyl in 20 mL of methanol by heating on a water-bath: the solution is clear.
(2) *Related substances* Proceed as directed in the Purity (6) under Carbamazepine: any spot other than the principal spot at the R_f value of about 0.9 does not appear.
Nitrogen between 6.8 and 7.3 % (Nitrogen Determination).

Imipramine hydrochloride $C_{19}H_{24}N_2 \cdot HCl$ [Same as the namesake monograph].

Indigo carmine $C_{16}H_8N_2Na_2O_8S_2$ [Special class].

Indigo carmine TS Dissolve 0.20 g of indigo carmine in water to make 100 mL. Use within 60 days.

Indometacin
$C_{19}H_{16}ClNO_4$[Sameasthenamesakemonograph].

Iodine I [Special class].

Iodine monobromide IBr Blackish brown crystals or masses. It dissolves in water, in ethanol (95), in diethyl ether, in carbon disulfide or acetic acid (100).
Melting point 40 °C
Storage Preserve in light-resistant glass containers, in a cold place.

Iodine-starch TS To 100 mL of starch TS, add 3 mL of dilute iodine TS

Iodine trichloride ICl_3 [Specialclass].

Iodine TS Dissolve 14 g of iodine in 100mL of a solution of potassium iodide (2 in 5), add 1mL of dilute hydrochloric acid, and dilute with water to make 1000mL (0.05 mol/L).
Storage Preserve in light-resistant containers.

Iodine TS, dilute To 1 volume of iodine TS, add 4 volumes of water.

0.0002 mmol/L Iodine TS Measure exactly 1 mL of 0.5 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.

Iodoethane C_2H_5I [Specialclass].

Iodomethane CH_3I [Special class].

Iron Fe Iron in the forms of strips, sheets, granules or wires.
Fe not less than 97.7 %.
It is attracted by a magnet.

Iron (III) chloride-acetic acid TS Dissolve 0.1 g of iron (III) chloride hexahydrate in diluted acetic acid (3 in 100) to make 100 mL.

Iron (III) chloride hexahydrate $FeCl_3 \cdot 6H_2O$[Specialclass].

Iron (III) chloride-iodine TS Dissolve 5 g of iron (III) chloride hexahydrate and 2 g of iodine in 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, anhydride Heat gradually 1.7 g of iron (III) chloride hexahydrate by direct application of flame, melt, and solidify. After cooling, dissolve the residue in 100 mL of chloroform, add 8mL of pyridine, and filter.

Iron (III) chloride TS Dissolve 9 g of iron (III) chloride hexahydrate in water to make 100 mL (1/3 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 hexahydrate TS.

Iron (III) chloride TS, dilute Dilute 2 mL of iron (III) chloride hexahydrate TS with water to make 100 mL. Prepare before use.

Iron (III) nitrate enneahydrate $Fe(NO_3)_3 \cdot 9H_2O$ [Special class].

Iron (III) nitrate TS Dissolve 1 g of iron (III) nitrate enneahydrate in hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 300 mL.

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.
Storage Preserve in tight containers in a cold place.

Iron (III) perchlorate hexabydrate $Fe(C1O_4) \cdot 6H_2O$, Hygroscopic, light purple crystals, and a solution in ethanol (99.5) (1 in 125) is clear and orange in color.

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 200mL of sulfuric acid and water to make 1000 mL.

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 0.1 g of sodium hydrogen sulfite in water to make 100 mL.

Iron (II) thiocyanate TS Add 3 mL of dilute sulfuric acid to 35 mL of water, and remove the dissolved oxygen by boiling the solution. Dissolve 1 g of iron (II) sulfate heptahydrate in this hot solution, cool, and then dissolve 0.5 g of potassium thiocyanate. When the solution is pale red in color, decolorize by, adding reduced iron, separate the excess of reduced iron by decanting, and preserve the solution with protection from oxygen. Do not use a solution showing a pale red color.

Iron-phenol TS Dissolve 1.054 g of ammonium iron (II) sulfate hexahydrate in 20 mL of water, add 1mL of sulfuric acid and 1mL of hydrogen peroxide (30), heat until effervescence ceases, and dilute with water to make 50 mL. To 3 volumes of this solution contained in a volumetric flask, add sulfuric acid, with cooling, to make 100 volumes, yielding the iron-sulfuric acid solution. Purify phenol by distillation, discarding the first 10 % and the last 5 %, and collect the distillate, with exclusion of moisture, in a dry, tared, glass-stoppered flask of about twice the volume of the phenol. Stopper the flask, solidify the phenol in an ice-bath, breaking the top crust with a glass rod to ensure complete crystallization, and after drying, weigh the flask. To the glass-stoppered flask, add 1.13 times the mass of phenol of the iron sulfuric acid solution, insert the stopper in the flask, and allow to stand without cooling but with occasional shaking, until the phenol is liquefied, then shake the mixture vigorously. Allow to stor a dark place for 16 to 24 hours. To the mixture, add diluted sulfuric acid (10 in 2l) equivalent to 23.5 % of its mass, mix well, transfer to dry glass-stoppered bottles, and preserve in a dark place, with protection from atmospheric moisture. Use within 6 months.

Iron-phenol TS, dilute Add 4.5 mL of water to 10mL of iron-phenol TS. Prepare before use

Iron powder Fe [Reduced iron, Special class].

Isatin See 2,3-indolinedione.

Isoamyl acetate $CH_3COOCH_2CH_2CH(CH_3)_2$ A clear and colorless liquid. Boiling point: about 140 °C.
Specific gravity d_{20}^{20}: between 0.868 and 0.879
Refractive index n_D^{20} about 1.395

Preserve in a light-resistant tight container.

Isoamyl alcohol See 3-methyl-1-butanol.

Isoamyl parahydroxybenzoate $C_{12}H_{16}O_3$ White crystalline powder, having a faint characteristic odor. It is very soluble in acetonitrile, in ethanol (95), in acetone or diethyl ether, and practically in soluble in water.
Melting point between 62 and 64 °C

Isobutanol See 2-methyl-1-propanol.

Isobutyl parahydroxybenzoate $C_{11}H_{14}O_3$, Colorless crystals or white crystalline powder. Odorless. Freely soluble in ethanol (95), in acetone or diethylether, and practically insoluble in water.
Melting point between 75 and 77 °C
Residue on ignition more than 0.1 %.
Content not less than 98.0 %.
Assay Proceed as directed in Assay under Ethyl Parahydroxybenzoate.

EachmL of 1 mol/L sodium hydroxide VS
= 194.23 mg of $C_{11}H_{14}O_2$

Isobutyl salicylate $C_{11}H_{14}O_3$ Colorless, clear liquid, having a characteristic odor.
Refractive index n_D^{20} between 1.506 and 1.511.
Specific gravity d_{20}^{20} between 1.068 and 1.073.
Boiling point between 260 and 262 °C.
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 calculate the amount of isobutyl salicylate by the area percentage method: not less than 97.0 %.

Operating conditions
Detector: A thermal conductivity detector.
Column: A column, about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with polythylene glycol 20 m for gas chromatography at the ratio of 10 %.
Column temperature: A constant temperature of about 220 °C.
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 the test solution composes about 60 % to 80 % of the full scale.
Time span of measurement: About 3 times as long as the retention time of isobutyl salicylate.

Isoniazid $C_6H_7N_3O$ [Same as the namesake monograph]

Isoniazid for Assay $C_6H_7N_3O$ [Same as the monograph isoniazid. When dried, it contains not less than 99.0 % of isoniazid ($C_6H_7N_3O$).].

Isoniazid TS Dissolve 0.1 g of isoniazid for Assay in a mixture of 50 mL of methanol and 0.12 mL of hydrochloric acid, and add methanol to make 200 mL.

Isonicotinic acid White, crystals or powder.
Melting point about 315 °C (decomposition).

Isonicotinic acid hydrazide See Isoniazid

Isooctane See octane, iso.

Isopropanol See 2-propanol.

Isopropanol for liquid chromatography See 2-propanol for liquid chromatography.

Isopropylamine See propylamine, iso.

Isopropylamine-ethanol TS To 20 mL of isopropylamine, add ethanol (99.5) to make 100 mL. Prepare before use.

Isopropyl 4-aminobenzoate $NH_2C_6H_4COOCH(CH_3)_2$ Pale brown crystals.
Melting point between 83 and 86 °C

Isopropyl p-aminobenzoate See isopropyl 4-aminobenzoate.

Isopropyl benzoate $C_6H_5COOCH(CH_3)_2$ A clear, colorless liquid, having a characteristic odor.
Referactive index n_D^{20} 1.490~1.498
Specific gravity d_{20}^{20} 1.008~1.016

Isopropylether See propylether, iso.
Isopropyl myristate C17H34O2 Colorless, clear, oily liquid, and odorless. Congeals at about 5 °C. Soluble in 90 % alcohol, miscible with many organic solvents or solid oils, soluble in water, glycerin or propylene glycol.
Refractive index n_D^{20} between 1.432 and 1.436.
Specific gravity d_{20}^{20} between 0.846 and 0.854.
Saponification value between 202 and 212.
Acid value not more than 1.
Iodine value not more than 1.
Residue on ignition not more than 0.1 % (1 g).

Isopropyl myristate for sterility test $C_{17}H_{34}O_2$, Transfer 100 mL of isopropyl myristate into acentrifuge tube, add 100 mL of twice-distilled water, and shake vigorously for 10 minutes. Then centrifuge at a rate of 1800 revolutions per minute for 20 minutes, separate the supernatant liquid (isopropyl myristatelayer), and determine the pH of the residual

water layer: less than 5.5. Treat isopropyl myristate which meets the requirements of pH determination as follows: 500 mL of isopropyl myristate, which has met the requirements of pH determination, is percolated through a 15 cm high layer of activated alumina, filled in a glass column 20 mm in diameter and 20 cm in length with a slightly positive pressure in order to facilitate adequate flow, and then sterilized by filtration.

Isopropyl para-bydroxybenzoate $C_{10}H_{12}O_3$, Odorless and colorless crystals, or white, crystalline powder. Freely soluble in ethanol (95), acetone or diethyl ether, and very slightly soluble in water.
Melting point between 84 and 86 °C
Residue on ignition more than 0.1 %.
Content not less than 99.0 %.
Assay Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

EachmL of 1 mol/L sodium hydroxide VS
= 180.20 mg of $C_{10}H_{12}O_3$

Isotonic sodium chloride injection [Same as the namesake monograph].

Kainic acid $C_{10}H_{15}NO_4.H_2O$ [Same as the namesake monograph].

Kanamycin sulfate $C_{18}H_{36}N_4O_{11}\cdot xH_2SO_4$ [Sameasthenamesakemonograph]

Karl Fischer TS See the Water Determination under the General Tests, Processes and Apparatus.

Kerosene It is mainly a mixture of hydrocarbons in the methane series, and a colorless, clear liquid, having not a disagreeable, characteristic odor.
Specific gravity d_{20}^{20} About 0.80.
Distilling range between 180 °C and 300 °C

Kininogen Produced by purifying from bovine plasma. Dissolve an appropriate amount of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 so that 10 mL of the solution contains 1 mg of kininogen, and use this solution as the sample solutioin. Perform the following tests with the sample solution: it meets the requirement of each test. (i) Immediately after this sample solution is prepared, add 0.1 mL of a solution of trichloroacetic acid (1 in 5) to 0.5 mL of the sample solution, shake, and centrifuge. To 0.5 mL of the supernatant liquid, add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution. Preceed with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the amount of kinin: kinin is not detected. (ii) Warm 0.5 mL of the sample slution at 30 ± 0.5 °C for 20 minutes and proceed as directed in (i): kinin is not detected. (iii) Perform the test with 0.5mL of the sample solution as directed in the Purity (2) under Kallidinogenase: the decomposition of bradykinin is not observed. (iv) To 0.5 mL of the sample solution add 0.5mL of 0.02 mol/L phosphate buffer solution. pH 8.0 containing 500 μg of crystal tyrpsin, previously warmed at 30 ± 0.5 °C for 5 mimutes, add 0.2 mL of a solution trichloroacetic acid (1 in 5), and shake. Then boil for 3 mimutes, cool in ice immediately, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 0.9mL of trichloro acetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution add trichloroacetic acid-gelatin-tris buffer solution to make 20 mL, then proceed as directed in (i), and determined the amount, BK, of kinin per well. Calculate the kinin-releasing activity per mg by the following equation: not less than 10μg bradykinin equivalent per mg.

Kinin-releasing activity per mg
(μg bradykinin equivalent/mg) = $B_k \times 0.0096$

Kininogen TS Dissolve a sufficient quantity of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 to prepare a solution having an ability in eachmL to release kinin corresponding to not less than 1 μg of bradykinin.

Lactic acid $CH_3CH(OH)COOH$ [Special class].

Lactic acid TS Dissolve 12.0 g of lactic acid in water to make 100mL.

Lactobionic acid $C_{12}H_{22}O_{12}$ Colorless crystals or white crystalline powder, having no odor.
Melting point between 113 and 118 °C
Purity. Dissolve 0.10 g of lactobionic acid in 10mL of a mixture of methanol and water (3:2), and perform the test with 10mL of this solution as directed in the Identification
(2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.
Residue on ignition notmorethan 28.5 %. (1 g)

Lactose See lactose monahydrate.

α-Lactose and β-lactose mixture (1 : 1) Use a mixture of lactose monohydrate and anhydrous lactose (3 : 5).

Lactose broth After, adding lactose monohydrate to ordinary broth in the ratio of 0.5 %, add about 12mL of bromothymol blue-sodium hydroxide TS to 1000mL of the medium. Then dispense portions of about 10mL into tubes for fermentation, and sterilize fractionally on each of three successive days for 15 to 30 minutes at 100 °C by using an autoclave, or sterilize by autoclaving for more than 20 minutes at 121 °C, and cool quickly by immersing in cold water.

Lactose broth, three times concentrated Add lactose monohydrate to ordinary broth prepared by using 330mL in place of 1000 mL of water in the ratio of 1.5 %, and prepare according to the method of preparation under lactose broth, with 25 mL volumes in tubes for fermentation.

Lactose broth, twice concentrated Add lactose monohydrate to ordinary broth prepared by using 500 mL in place of 1000 mL of water in the ratio of 1.0 % and prepare according to the method of preparation under lactose broth.

Lactose monobydrate $C_{12}H_{22}O_{11}\cdot H_2O$ [Same as the monograph Lactose].

Lactose substrate solution Dissolve 6.0 g of lactose in disodium hydrogen phosphate-citric acid buffer solution, pH 4.5, to make 100 mL.

Lauromacrogol [Same as the namesake monograph]

Lead acetate See lead (II) acetate trihydrate.

Lead acetate paper Usually, immerse strips of filter paper, 6 cm ~ 8 cm in size, in lead acetate TS, drain off the excess liquid, and dry the paper at 100 °C, avoiding contact with metals.

Lead acetate TS To 9.5 g of lead (II) acetate trihydrate, add freshly boiled and cooled water to make 100mL. Preserve in tightly stoppered bottles (0.25 mol/L).

Lead dioxide See lead (IV) oxide.

Lead (II) acetate trihydrate $Pb(CH_3COO)_2\cdot 3H_2O$ [Specialclass].

Lead (II) nitrate $Pb(NO_3)_2$[Special class].

Lead (II) oxide PbO A dark brown to black-brown, powder or granules.
Identification. A supernatant liquid of a solution in dilute acetic acid (1 in 100) responds to Quality Tests (3) for lead salt.

Lead (IV) oxide PbO_2 [Special class].

Lead monoxide See lead (II) oxide.

Lead nitrate See lead (II) nitrate.

L-Leucine $C_6H_{13}NO_2$ [Sameasthenamesakemonograph]

Levothyroxine sodium $C_{15}H_{11}I_4NNaO_4\cdot xH_2O$ [Same as the namesake monograph].

Limonene $C_{10}H_{16}$ Clear and colorless liquid, having a specific perfume and a bitter taste.
Refractive index n_D^{20} between 1.427 and 1.474.
Specific gravity d_{20}^{20} between 0.841 and 0.846.
Melting point between 176 and 177 °C.
Purity Related substances Dissolve 0.1 g of limonene in 25mL of exane and use this solution as the test solution. Perform the test with 2 μL of the test 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 limonene: It is not less than 97.0 %.

Operating conditions

Proceed the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Measure 1 mL of limonene, add hexane to make 100 mL, and adjust the detection sensitivity so that the peak height of limonene obtained from 2 μL of this solution is 40 % to 60 % of the full scale.

Time span of measurement: About 3 times as long as the retention time of limonene after the solvent peak.

Liothyronine sodium $C_{15}H_{11}I_3NNaO_4$ [Same as the namesake monograph]

Liquid parafin See paraffin, liquid.

Lithium acetate dehydrate $CH_3COOLi\cdot 2H_2O$ Colorless crystals.
Dilute acetic acid insoluble substances To 40.0 g of lithium acetate dehydrate add 45mL of water, heat in a water bath to dissolve, cool, then dissolve in dilute acetic acid, and filter by suction. Wash the filter with water, dry the filter at 105 ± 2 °C. for 1 hour, and weigh the mass of the residue after cooling:not more than 0.0025 %.
Content not less than 97.0 %.
Assay Weigh accurately 0.3 g of Lithium acetate dehydrate, add exactly 50mL of acetic acid (100) and exactly 5mL of acetic anhydride, dissolve by heating in a water bath, and titirate with 0.1 mol/L perchloric acid VS after cooling (potentiometirc titration). Perform a balnk determination in the same manner, and make any necessary correction.

EachmL of 1 mol/L perchloric acid VS
= 10.201 mg of $CH_3COOLi\cdot 2H_2O$

Lithium chloride LiCl.

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 paper, blue [Litmus paper, Blue litmus paper].

Litmus paper, red [Litmus paper, Red litmus paper].

Sodium choride	9.0 g
Potassium choride	0.42 g
Calcium chloride dehydrate	0.24 g
Magnesium chloride	0.2 g
Sodium bicarbonate	0.5 g
Dextrose	0.5g
Water, freshly distilled with a hard-glass apparatus	a sufficient quantity
	To make 1000 mL

Locke-Ringer's TS Prepare before use. The constituents except dextrose and sodium hydrogen carbonate can be made up in concentrated stock solutions, stored in a dark place, and diluted before use.

Loganin for assay $C_{17}H_{26}O_{10}$ White, crystals of crystalline powder. Solunle in water, sparingly soluble in methanol, and very slightly soluble in ethanol(99.5).
Melting point between 211 and 227 °C
Absorbance $E^{1\%}_{1\,cm}$ (235 nm) : 275~303 (dried in a desiccator (silica gel) for 24 hours, 5 mg, methanol, 500 mL).
Purity Related substances—Dissolve 2 mg of loganin for assay in 5mL of the mobile phase, and use this solution as the sample solution. Pipet 1mL of this solution, add the mobile phase to make exactly 100mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of loganin is not larger than the peak area of loganin from the standard solution.

Operating conditions.
Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 mm in particle diameter).
Column temperature: A constant temperature of about 50 °C.
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: When the procedure is run with 10 mL of the standard solution under the above operating conditions, the number of the oretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 10 mL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5 %.

Lysate reagent A lyophilized product obtained from amebocyte lysate of horsehoe crab (Limulus polyphemus or Tachypleus tridentatus). Ameobocyte lysate preparations which do no react to -glucans from a mebocytelysate or byinhibiting the G factor reactin system of a mebocytelysate.

Lysate TS Dissolve a lysate reagent in water ofr bacterial endotoxins test, or in a suitable buffer, by gentle stirring.

L-Lysine hydrochloride $C_6H_{14}N_2O_2{\cdot}HCl$ [Same as the namesake monograph].

Magnesia TS Dissolve 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride in 65mL of water, add 35mL of ammonia TS, allow the mixture to stand for a few days in tightly stoppered bottles, and filter. If the solution is not clear, filter before use.

Magnesium Mg [Special class].

Magnesium buffer solution pH 9 Mix 3.1 g of boric acid and 500 mL of water in a 1000 mL volumetric flask, add 21 mL of 1 N sodium hydroxide and 10 mL of 0.1 M magnesium chloride, dilute with water to volume, and mix.

Magnesium chloride See magnesium chloride hexahydrate.

Magnesium chloride hexahydrate $MgCl_2{\cdot}6H_2O$ [Special class].

Magnesiam nitrate hexahydrate $Mg(NO_3)_3{\cdot}6H_2O$ [Special class].

Magnesium oxide MgO [Special class].

Magnesium powder Mg [Special class].

Magnesiam sulfate See magnesium sulfate heptahydrate.

Magnesium sulfate heptahydrate $MgSO_4{\cdot}7H_2O$ [Special class].

Magaesium sulfate TS Dissolve 12 g of magnesium sulfate hexahydrate in water to make 100 mL (0.5 mol/L).

Malachite green See malachite green oxalate.

Malachite green TS Dissolve 1 g of malachite green oxalate in 100 mL of acetic acid(100).

Malachite green oxalate $C_{52}H_{54}N_4O_{12}$[Malachite green(oxalate), Special class].

Maleic acid $C_4H_4O_4$ [Specialclass]. A white crystalline powder.
Identification. Determine the infrared absorption spectrum of maleic acid as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry : it exhibits absorption at the wave numbers 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 See maitose monohydrate.

Manganese dioxide MnO_2 [First class].

D-Mannitol $C_6H_{14}O_6$ [Same as the monographD-Mannitol].

D-Mannose $C_6H_{12}O_6$ White crystal or crystalline powder. It is very soluble in water.
Melting point: about 132 °C (with decomposition).
Optical rotation $[\alpha]_D^{20}$: +13.7 ~+14.7°(4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm).

***d*- mannosaminehydrochloride** $C_6H_{13}NO_5 \cdot HCl$ White powder.
Melting point: about 168 °C (with decomposition).
Optical rotation $[\alpha]_D^{20}$: −4.2 ~−3.2°(0.4 g, water, 20 mL, 100 mm).

Mayer's TS Dissolve 1.358 g of mercury (II) chloride in 60mL of water. Dissolve 5 g of potassium iodide in 10mL of water. Mix both solutions, and add water to make 100mL.

Meat extract Beef extract or an extract of a similar grade.

Meglumine $C_7H_{17}NO_5$ [same as the namesake monograph]

Mentha oil [Same as the monograph in Part II].

Menthol $C_{10}H_{20}O$ [Same as the monograph in Part II dl-Menthol or l-Menthol].

Mercaptoacetic acid $HSCH_2COOH$ [Special class]. Place in an ampule, andpreserve in a dark, cold place. Do not use afters toring for a long period.

Mercaptopurine $C_5H_4N_4S.H_2O$ [Same as the namesake monograph].

Mercuric acetate See mercury (II) acetate

Mercuric acetate TS for nonaqueous titration See mercury (II) acetate TS for nonaqueous titration.

Mercuric bromide See mercury (II) bromide.

Mercuric bromide paper See mercury (II) bromide paper.

Mercuric chloride See mercury (II) chloride.

Mercuric chloride TS See mercuric chloride TS.

Mercuric nitrate TS See mercury (II) nitrate TS.

Mercury Hg [Special class].

Mercury (II) acetate $Hg(CH_3COO)_2$ [Special class]. White crystals or crystalline powder.
Identification. (1) Dissolve 1 g of mercury (II) chloride in 1 mL of diluted nitric acid (1 in 7), add 20mL of water, and use this as the sample solution. To 10mL of the sample solution add 0.8 mL of iron (III) chloride TS: a red-brown color is developed.
(2) To 10 mL of the sample solution obtained in (1) add 2 mL of potassium iodate TS: a red precipitate is produced. Preserve in a light-resistant tight container.

Mercury (II) acetate TS for nonaqueous titration Dissolve 5 g of mercury (II) acetate in glacial acetic acid for non-aqueous titration 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 solution, 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 size, 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 100mL (0.25 mol/L).

Mercury (II) nitrate TS Dissolve 40 g of yellow mercury (II) oxide in a mixture of 32 mL of nitric acid and 15 mL of water (4 mol/L). Preserve in light-resistant, glass-stoppered bottles.

Mercury (II) oxide, yellow HgO [Special class]. Preserve in light-resistant containers.

Mercury (II) sulfate TS Mix 5 g of yellow mercury (II) oxide with 40mL of water, and add slowly 20mL of sulfuric acid while stirring, then, add another 40mL of water, and stir until completely dissolved.

Metanil yellow $C_{18}H_{14}N_3NaO_3S$, A yellow-brown powder. Sparingly soluble in water, and very slightly soluble in ethanol (95) or *N,N*-dimethylformamide.
Transition interval (color change): pH 1.2 (red) - 2.3 (yellow).

Metanil yellow TS Dissolve 0.1 g of metanil yellow in 200 mL of *N,N*-dimethylformamide.

Metaphosphoric acid HPO_3 A colorless, deliquescent stick or masses.
Identification. (1) Dissolve 1 g of metaphosphoric acid in 50 mL of water, and use this as the sample solution. To 10 mL of the sample 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 sample 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, and use within 2 days.

Methanesulfonic acid CH_3SO_3H Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, ethanol (95) or diethyl ether.
Congealing point between 15 °C and 20 °C
Specific gravity d_{20}^{20} between 1.483 and 1.488
Content not less than 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).

EachmL of 1 mol/L sodium hydroxide VS
= 96.11 mg of CH_3SO_3H

Methanesulfonic acid TS To 35mL of methanesulfonic acid, add 20mL 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, anhydrous CH_4O To 1000mL of methanol add 5 g of magnesium powder. If necessary, add 0.1mL of mercury (II) chloride TS to start the reaction. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate protecting from moisture. Water content permL is not more than 0.3 mg.

Methanol for Karl Fischer method See the Water Determination under the General Tests, Processes and Apparatus.

Methanol, purified Distil methanol before use.

Methionine $C_5H_{11}NO_2S$ [Same as the monograph L-Methionine].

2-Methoxyethanol for Karl Fischer method See the Water Determination under the General Tests, Processes and Apparatus.

4-Methoxybenzaldehyde $C_8H_8O_2$, Clear, colorless to light yellow liquid. Miscible with ethanol (95) or diethyl ether, and practically insoluble in water.
Specific gravity d_{20}^{20} between 1.123 and 1.129.
Content not less than 97.0 %.
Assay Weigh accurately about 0.8 g of *p*-anisaldehyde, add exactly 7.5 mL ofh ydroxy lamine TS, shake well, allow to stand for 30 minutes, and titrate with 0.5mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS) until the color of the solution changes from blue through green to yellow-green. Perform a blank determination and make any necessary correction.

EachmL of 0.5 mol/L hydrochloric acid VS
= 68.08 mg of $C_8H_8O_2$

4-Methoxybenzaldehyde-acetic acid TS To 0.5mL of 4-methoxybenzaldehyde, add glacial acetic acid to make 100 mL.

4-Methoxybenzaldehyde-sulfuric acid TS To 9mL of ethanol (95), add 0.5mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix thoroughly

2-Methoxy-4-methylphenol $C_8H_{10}O_2$ Colorless to pale yellow liquid. Miscible with methanol and with ethanol (99.5), and slightly soluble in water. Congealing point: 3 -8 °C.
Identification. Determine the infrared absorption spectrum of 2-methoxy-4-methylphenol as directed in the ATR method under Infrared Spectrophotometry : it exhibits absorption at the wave numbers of about 1511cm^{-1}, 1423cm^{-1}, 1361cm^{-1}, 1268cm^{-1}, 1231cm^{-1}, 1202cm^{-1}, 1148cm^{-1}, 1120cm^{-1}, 1031cm^{-1}, 919cm^{-1}, 807cm^{-1} and 788 cm^{-1}.
Purity Related substances. Perform the test with 0.2mL of 2-methoxy-4-methylphenol as directed under Gas Chromatography according to the following conditions. Determine each peak by the automatic integration method: the total area of the peaks other than the peak of 2-methoxy- 4-methylphenol is not more than 3.0 %.

Operating conditions
Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polymethylsiloxane for gas chromatography in 0.25 to 0.5 mm in 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.
Injection port temperature: 200 °C.
Detector temperature: 250 °C.
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 100mL, and use this solution as the solution for system suitability test. Proceed with 1 μL of the solution for system suitability test under the above operating conditions, the symmetry factor of the peak of 2-methoxy-4-methylphenol is not more than 1.5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 2-methoxy-4-methylphenol is not more than 2.0 %.

1-Methoxy-2-propanol $C_4H_{10}O_2$ A colorless, clear liquid.
Clarity of solution To 5mL of 1-Methoxy-2-propanol add 20mL of water and mix: the solution os clear.
Refraction index n_D^{20} 1.402-1.405
Specific gravity d_{20}^{20} 0.920-0.925
Water not more than 0.5 % (5g).
Content not less than 98.0 % (gas Chromatography).
Assay Proceed as directed under the Gas Chromatography using the area percentage method according to the following condions:

Operating conditions
Detector: Thermal conductivity detector
Column: A glass colum about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150-180 μm) coated with polyethylene glycol 20 m for gas chromatography in 20 %.
Column temperature: A constant temperature of about 90 °C
Carrier gas: Helium
Flow rate: Adjust to a constant flow rate of 20mL per minute.

***p*-Methyl aminophenol sulfate** See 4-methyl aminophenol sulfate.

4-Methyl aminophenol sulfate $(HOC_6H_4NHCH_3)_2.H_2SO_4$ White to pale yellow or very pale grayish white, crystals or crystalline powder.
Melting point : about 260 °C (with decomposition).

p-Methyl aminophenol sulfate TS See 4-methyl aminophenol sulfate TS.

4-Methyl aminopbenol sulfate TS Dissolve 0.35 g of 4-methyl aminophenol sulfate and 20 g of sodium hydrogen sulfite in water to make 100 mL. Prepare before use.

2-Methylaminopyridine $C_6H_8N_2$, A pale yellow liquid.
Specific gravity d_{20}^{20} between 1.050 and 1.065,
Boiling point between 200 °C and 202 °C,
Water less than 0.1 %.

2-Methylamino pyridine for Karl Fischer method See the Water Determination under the General Tests, Processes and Apparatus.

Methyl behenate $C_{23}H_{46}O_2$, White, odorless and tasteless, scaly crystals or powder. Dissolves in acetone, in diethyl ether or chloroform.
Melting point 54 °C
Saponification value between 155.5 and 158.5.

Methyl benzoate $C_6H_5COOCH_3$, A clear, colorless liquid.
Refractive index n_D^{20} between 1.515 and 1.520.
Specific gravity d_{20}^{20} between 1.087 and 1.095.
Purity Dissolve 0.1mL of methyl benzoate in the mobile phase in the Assay under Thiamine Hydrochloride to make 50 mL. Perform the test as directed under the Liquid Chromatography with 10 μL of this solution according to the Assay under Thiamine Hydrochloride. Measure each peak area of the solution by the automatic integration method in a range about twice the retention time of the main peak, and calculate the amount of methyl benzoate by the area percentage method: it is less than 99.0 %.

Methyl benzoate for estriol test $C_8H_8O_2$, A clear, colorless liquid, having a characteristic odor.
Refractive index n_D^{20} between 1.515 and 1.520
Specificgravity d_{20}^{20} between 1.087 and 1.095
Acid value more than 0.5.

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} between 1.524 and 1.529
Specific Optical Rotation $[\alpha]_D^{20}$ between +37 and +41o (50 mm)

Content not less than 98.0 %.
Assay Perform the test with exact 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, and calculate the amount of D-(+)-α-methylbenzylamine.

Operating conditions
Detector: A hydrogen flame-ionization detector.
Column: A glass column, about 3 mm in inside diameter and about 2 m in length, having siliceous earth for gas chromatography (180 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 percent, respectively.
Column temperature: A constant temperature of about 140 °C
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 1mL of pyridine. Proceed with 0.6 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pyridine and D-(+)-α-methylbenzylamine in this order with the resolution between their peaks being less than 3.
Time span of measurement: About three times as long as the retention time of D-(+)-α-methylbenzylamine.

Methyl cellosolve See 2-methoxyethanol

Methylene blue $C_{16}H_{18}ClN_3S \cdot {}_3H_2O$ [Special class].

Methylne blue-sulfuric acid-sodium dihydrogen phosphate TS To 30 mL of a solution of methylene blue (1 in 1000) add 500 mL of water, 6.8 mL of sulfuric acid and 50 g of sodium dihydrogen phosphate dehydrate, dissolve, and add water to make 1000 mL.

Methylene blue TS Dissolve 0.1 g of methylene blue in water to make 100 mL. Filter if necessary.

***dl*-Methylephedrine hydrochloride** $C_{11}H_{17}NO \cdot HCl$ [Same as the namesake monograph]

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-boric acid TS Add 0.5 g of methyl orange and 5.2 g of boric acid in 500 mL of water, and dissolve by warming on a water-bath. After cooling, wash this solution with three 50 mL volumes of chloroform.

Methyl orange TS Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.

Methyl orange-xylenecyanol FF TS Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500mL of dilute ethanol.

Methyl parahydroxybenzoate $HOC_6H_4COOCH_3$ [Same as the namesake monograph in part II, Methylparaben]

Methyl prednisolone $C_{22}H_{30}O_5$ [Same as the namesake monograph]

Methyl red $C_{15}H_{15}N_3O_2$ [Special class].

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, and filter if necessary. Preserve in light-resistant containers.

Methyl red TS Dissolve 0.1 g of methyl red in 100mL of ethanol (95), and filter, if necessary.

Methyl red TS, dilute Dissolve 0.025 g of methyl red in 100 mL of ethanol (99.5), and filter, if necessary. Prepare before use.

Methyl red TS for acid or alkali test To 100 mg of methyl red, add 7.4 mL of 0.05 mol/L sodium hydroxide VS or 3.7 mL of 0.1 mol/L sodium hydroxide VS, triturate to dissolve in a mortar, and add freshly boiled and cooled water to make 200 mL. Preserve in light-resistant, glass-stoppered bottles.

Methyl salicylate $C_8H_8O_3$ [Same as the namesake monograph].

Methyl testosterone $C_{20}H_{30}O_2$ [Same as the namesake monograph].

Methylthymol blue $C_{37}H_{43}N_2NaO_{13}S$

Methylthymol blue-potassium nitrate indicator Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate until the mixture becomes homogeneous.
Sensitivity When 0.02 g of methylthymol blue-potassium nitrate indicator is dissolved in 100 mL of 0.02 mol/L sodium hydroxide VS, the solution is slightly blue in color. On adding 0.05 mL of 0.01 mol/L barium chloride VS to this solution, the solution shows a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L disodium ethylenediamineteraacetate VS, it becomes colorless.

Methylthymol blue-sodium chloride indicator Mix 0.25 g of methylthymol blue and 10 g of sodium chloride, and grind to homogenize.

Methyl yellow $C_{14}H_{15}N_3$ [Specialclass].

Methyl yellow TS Dissolve 0.1 g of methyl yellow in 200mL of ethanol (95).

3-Methyl-1-butanol $C_5H_{12}O$ [Specialclass].

3-Methyl-1-phenyl-5-pyrazolone $C_{10}H_{10}N_2$ [Special class].

2-Methyl-1-propanol $(CH_3)_3CHCH_2OH$ [Special class].

4-Methyl-2-pentanone $CH_3COCH_2CH(CH_3)_2$ [Special class].

Milk casein See casein, milk.

Molybdenum trioxide See molibdenum (III) trioxide.

Molybdenum trioxide-citric acid TS See molybdenum (III) trioxide-citric acid TS.

Monoethanolamine See 2-Aminoethanol.

Morpholin C_4H_9NO [Special class].

Murexide $C_8H_8N_6O_6$ Red-purple powder. Practically insoluble in water, in ethanol (95) or diethyl ether.
Purity Clarity of solution Dissolve 0.01 g of murexide in 100mL of water: the solution is clear.
Residue on ignition more than 0.10 % (1 g).
Sensitivity Dissolve 0.010 g of murexide in 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, and add water to make 100 mL, and use this solution as the test solution. Separately, add 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, to 5 mL of diluted standard calcium solution (1 in 10), add water to make 25 mL, and render the solution to pH 11.3 with sodium hydroxide TS., add 2 mL of the test solution and water to this solution to make 50 mL: a red-purple color develops.

Murexide-sodium chloride indicator Prepared by mixing 0.1 g of murexide and 10 g of sodium chloride, and grinding to get homogeneous.
Storage Preserve in light-resistant containers.

Naphazoline nitrate $C_{14}H_{14}N_2.HNO_3$ [Same as the namesake monograph].

Naphthalene $C_{10}H_8$ [Special class].

2-Naphthalenesulfonic acid See 2-Naphthalene sulfonic acid Hydrate

2-Naphthalenesulfonic acid Hydrate $C_{10}H_8O_3S\cdot H_2O$ White to pale yellowish white powder. Very soluble in water. In methanol or in ethanol, and sparingly soluble in diethyl ether or in chloroform.
Water: Between 7.0 % and 11.5 % (0.5 g, volumetric titration, direct titration).
Content : not less than 95.0 %, calculated on the anhydrous basis.
Assay Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid, dissolve in 30mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue IS). Perform a blank determination and make any necessary correction.

EachmL of 0.1 mol/L soduim hydroxide VS
= 20.82 mg of $C_{10}H_8O_3S$

1-Naphthol $C_{10}H_7OH$ [Special class].Preserve inlight - resistant containers.

2-Naphthol $C_{10}H_7OH$ [Special class].Preserve inlight - resistant containers.

α-Naphthol See 1-naphthol.

β-Naphthol See 2-naphthol.

***p*-Naphtholbenzeine** $C_{27}H_{20}O_3$ [Special class].

α-Naphtholbenzeine See *p*-naphthol benzeine.

***p*-Naphtholbenzeine TS** Dissolve 0.2 g of p-naphtholbenzeine in acetic acid (100) to make 100 mL.
Purity Clarity and color of solution Dissolve 0.1 g of *p*-naphtholbenzeine in 100 mL of ethanol (95): the solution is red in color and clear.
Sensitivity Add 100mL of freshly boiled and cooled water to 0.2 mL of a solution of p-naphtholbenzeine in ethanol (95) (1 in 1000), and add 0.1mL of 0.1 mol/L sodium hydroxide VS: a green color develops. Add subsequently 0.2 mL of 0.1 mol/L hydrochloric acid VS: the color of the solution changes to yellow-red.

α-Naphthol benzeine TS See p-naphtholbenzeineTS.

1-Naphthol TS Dissolve 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in water to make 100 mL. In this solution, dissolve 1 g of 1-naphthol. Prepare before use.

2-Naphthol TS Dissolve 1 g of 2-naphthol in sodium carbonate TS to make 100mL. Prepare before use.

α-Naphthol TS See 1-naphthol TS.

β-Naphthol TS See 2-naphthol TS.

1-Naphthylamine $C_{10}H_7NH_2$ [Special class]. Preserve inlight - resistant containers.

α-Naphthylamine See l-naphthylamine.

Nessler's TS Dissolve 10 g of potassium iodide in 10 mL of water, and add, with stirring, a saturated solution of mercury (II) chloride until a slight red precipitate remains undissolved. To this mixture,add a solution prepared by dissolving 30 g of potassium hydroxide in 60 mL of water, then, add 1 mL of the saturated solution of mercury (II) chloride and water to make 200 mL. Allow the precipitate to settle, and use the supernatant liquid. A 2 mL volume of this solution, when, added to 100mL of a solution of ammonium chloride (1 in 300000), produces at once a yellow-brown color.

Neutral red $C_{15}H_{17}N_4Cl$ [Special class].

Neutral red TS Dissolve 0.1 g of neutral red in acetic acid (100) to make 100mL.

Nicotinamide $C_6H_6N_2O$ [Same as the namesake monograph]

β-Nicotinamide-adenine dinucleotide (β-NAD) $C_{21}H_{27}N_7O_{14}P_2$
Content : not less than 94.5 %.
Assay. Weigh accurately about 25 mg of b-nicotinamide-adenine dinucleotide, oxidized form, and dissolve in water to make exactly 25 mL. Pipet 0.2mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10mL, and use this solution as the sample solution. Determine the absorbances, A_T and A_B, of the sample solution and0. 1 mol/L phosphate buffer solution, pH 7.0, at 260 nm as directed under Ultraviolet-visible Spectrophotometry, using water as the blank.

$$\text{Amount (mg) of } C_{21}H_{27}N_7O_{14}P_2 = \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_T - A_B) \times 25$$

β-Nicotinamide adenine dinucleotide (β-NAD) TS Dissolve 40 mg of β-nicotinamide adenine dinucleotide, oxidized form in 10 mL of water. Prepare before use.

Nifedipine $C_{17}H_{18}N_2O_6$ [Same as the namesake monograph].

Ninhydrin $C_9H_6O_4$ [Specialclass].

Ninhydrin-ascorbic acid TS Dissolve 0.25 g of ninhydrin and 0.01 g 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 500mL 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. In 100mL of this solution dissolve 0.2 g of ninhydrin.

Ninhydrin-stannous chloride TS See ninhydrin-tin(II) chloride TS.

Ninhydrin-sulfuric acid TS Dissolve 0.1 g of ninhydrin in 100mL of sulfric acid. Prepare before use.

Ninhydrin-tin (II) chloride TS Dissolve 21.0 g of citric acid in water to make 200 mL, adjust the pH to 5.6 ± 0.2 by, adding sodium hydroxide TS, add water to make 500 mL, and dissolve 1.3 g of tin (II) chloride. To 50mL of the solution, add 50mL of a 2-methoxethanol solution of ninhydrin (2 in 50). Prepare before use.

0.2 % Ninhydrin-water saturated 1-butanol TS Dissolve 2 g of ninhydrin in 1-butanol saturated with water to make 1000 mL

Ninhydrin TS Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

Nitric acid HNO_3 [Special class, Concentration: betweem 69 % and 70 %, Density: about 1.42 g/mL].

Nitric acid, dilute Dilute 10.5 mL of nitric acid with water to make 100 mL (10 percent).

Nitric acid, fuming [Special class, Concentration : less than 97percent, Density: 1.52 g/Ml].

2,2',2″-Nitrilotriethanol $(CH_2CH_2OH)_3N$ [First class].

***p*-Nitroaniline** See 4-nitroaniline.

4-Nitroaniline $O_2NC_6H_4NH_2$ Yellow to yellowish-red crystals or crystalline powder.
Melting point : 147 . 150 °C.
Preserve in a light-resistant tight container.

***p*-Nitroaniline-sodium nitrite TS** See 4-nitro aniline-sodium nitrite.

4-Nitroaniline-sodium nitrite TS To 90 mL of a solution of 0.3 g of 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS, add 10 mL of a solution of sodium nitrite (1 in 20), and mix well. Prepare before use.

o-Nitrobenzaldehyde See2-nitrobenzaldehyde.

2-Nitrobenzaldehyde $O_2NC_6H_4CHO$, Pale yellow crystals or crystalline powder.
Melting point between 42 °C and 44 °C.

Nitrobenzene $C_6H_5NO_2$[Special class].

***p*-Nitrobenzenediazonium chloride TS** See 4-nitrobenzene diazonium chloride TS.

4-Nitrobenzenediazoaium chloride TS Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, add 1.5 mL of water, and then, add a solution prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while cooling in an ice-bath. Prepare before use.

***p*-Nitrobenzenediazonium chloride TS for spraying** See 4-nitrobenzenediazonium chloride TS for spraying.

4-Nitrobenzenediazonium chloride TS for spraying Dissolve 0.4 g of 4-nitroaniline in 60 mL of 1 mol/L hydrochloric acid TS, and add, while cooling in an ice-bath, sodium nitrite TS until the mixture turns potassium iodide-starch paper to blue in color. Prepare before use.

4-Nitrobenzenediazonium fluoroborate $O_2NC_6H_4N_2BF_4$ Pale yellowish white, almost odorless powder. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly solute in ethanol (95) and in chloroform.
Identification Add 1mL each of a solution of phenol(1 in 1000) and sodium hydroxide TS to 10 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 1000): a red color develops.
Melting point about 148 °C (with decomposition).
Loss on drying not more than 1.0 % (1 g silica gel, 2 hours).

***p*-Nitrobenzene diazonium fluoroborate** See 4-nitrobenzenediazoniumfluoroborate.

***p*-Nitrobenzoyl chloride** See 4-nitrobenzoylchloride

4-Nitrobenzoyl chloride $O_2NC_6H_4OCl$ Light yellow crystals.
Melting point between 70 °C and74 °C
Content not less than 98.0 %.
Assay Weigh accurately about 0.5 g of 4-nitrobenzoyi chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash with water, dry at 105 °C to a constant mass, and weigh. The mass of 4-nitrobenzoyl chloride, multiplied by 1.107, represents the mass of 4-nitrobenzoylchloride ($C_7H_4ClNO_3$).

***p*-Nitrobenzyl chloride** See 4-nitrobenzyl chloride.

4-Nitrobenzyl chloride $O_2NC_6H_4CH_2Cl$ Light yellow crystals or crystalline powder. Soluble in ethanol (95).
Melting point between 71 °C and 73 °C
Content not less than 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 (95) to make 100 mL, and heat in a water-bath under a reflex condensor for 1 hour. After cooling, filter the precipitate with a glass filter, wash with water, dry at 105 °C to a constant mass, and weigh. The mass of the precipitate represents the amount of silver chloride (AgC1: 143.32).

Amount (mg) of 4-nitrobenzyl chloride = amount (mg) of silver chloride (AgCl: 143.32) × 1.197

4-(4-Nitrobenzyl)pyridine $C_{12}H_{10}N_2O_2$ Pale yellow, crystaliine powder. Freely soluble in acetone, and soluble in ethanol (95).
Melting point between 69 and 71 °C

***p*-Nitrobenzyl Bromide** $NO_2C_6H_4CH_2Br$ Almost white to pale yellow crystals, darkening on exposure to light. Practically insoluble in water; freely soluble in alcohol, in ether, and in glacial acetic acid. Store in tight, light-resistant containers.
Meltion point between 98 and 100 °C.
Solubility Separate 200 mg portions yield clear solutions in 5 mL of alcohol and in 5 mL of glacial acetic acid.

Nitroethan $C_2H_5NO_2$
Density 1.048 . 1.053 g/cm3 (20 ℃)
Water not more than 0.1 %.

Nitrogen [Same as the namesake monographin 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 metal cylinder may be used.

Nitromethane CH_3NO_2[Special class].

o-Nitrophenyl-β-D-galactopyranoside See 2-nitrophenyl-β-D-galactopyranoside.

2-Nitrophenyl-β-D-galactopyranoside $C_{12}H_{15}NO_8$ A white crystalline powder. Odorless. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.
Melting point between193 °C and 194 °C
Purity Clarity and color of solution A solution of 2-nitrophenyl-β-D-galactopyranoside (1 in 100) is clear and colorless.
Loss on drying more than 0.1 % (0.5 g, 105 °C, 2 hours).
Content not less than 98.0 %.
Assay Weigh accurately about 0.05 g of o-nitrophenyl-β-D-galactopyranoside, previously dried, 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 Spectrophotometry.

Amount (mg) of 2-nitrophenyl-β-D-galactopyranoside

$$= \frac{A}{133} \leftarrow 25{,}000$$

1-Nitroso-2-naphthol $C_{10}H_7NO_2$ A yellow-brawn to red-brown crystalline powder.
Melting point : 106 ~ 110 °C.
Preserve in a light-resistant tight container.

1-Nitroso-2-naphthol TS Dissolve 0.06 g of 1-nitroso-2-naphthol in 80 mL of acetic acid (100), and add water to make 100 mL.

α-Nitroso-β-naphthol See 1-nitroso-2-naphthol.

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 metal cylinder.

NN Indicator Mix 0.5 g of 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid with 50 g of anhydrous sodium sulfate, and triturate until the mixture becomes homogeneous.

n-Octane C_8H_{18}
Specific gravity d_4^{20} between 0.700 and 0.705
Purity Perform the test with 2 μL of n-octane as direced under the Gas Chromatography according to the conditions in the Assay under Hydroxy propyl methyl cellulose 2208. Measure each peak area by the automatic integration method, and calculate the amount of n-octane by the area percentage method: less than 99.0 %.

Octane, iso A colorless liquid. Practically insoluble in water. Miscible with diethyl ether or chloroform.
Purity Determine the absorbances of isooctane at 230 nm, 250 nm, and 280 nm as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank solution: these values are more than 0.050, 0.010, and 0.005, respectively.

1-Octanol $CH_3(CH_2)_6CH_2OH$ [Special class].

1-Octyl alcohol Seel-octanol.

n-Octylbenzen $C_{14}H_{22}$, Clear and colorless liquid, having a characteristic odor.
Specific gravity d_4^{20} 0.854 - 0.863
Distillation test between 263 °C and 265 °C less than 95 vol %.

Olive oil [Same as the name sake monograph in PartII].

Orcine $C_7H_3O_2$, White to light red-brown crystals or crystalline powder, having an unpleasant, sweet taste. It turns to red in color when oxidized in air. Soluble in water, in ethanol (95), or diethyl ether.
Meting point between 107 and 110 °C

Orcine-ferric chloride TS See orcine-iron (III) chloride TS.

Orcine-iron (III) chloride TS Dissolve 10 mg of orcine in 1mL of a solution of iron (III) chloride hexahydrate in hydrochloric acid (1 in 1000). Prepare before use.

Ordinary agar medium Dissolve 25 to 30 g of agar in 1000 mL of ordinary broth with the aid of heat, add water to make up for that loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the liltrate, and sterilize by autoclaving. When powdered agar is used, l5 to 20 g of it is dissolved.

Ordinary broth Dissolve 5 g of beef extract and 10 g of peptone in 1000mL of water by gentle heating. Adjust the pH of the mixture between 6.4 and 7.0 after sterilization, cool, add water to make up for that lost, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121 °C

Oxalic acid See oxalic acid dihydrate.

Oxalic acid dehydrate $H_2C_2O_4 \cdot 2H_2O$ [Special class].

Oxalic acid TS Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

Oxygen O_2 [Same as the namesake monograph]

8-Oxyquinoline See 8-quinolinol.

Palladium chloride See palladium (II) chloride.

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 in 500 mL of 0.25 mol/L sulfuric acid TS, b heating if necessary, cool, and add 0.25 mol/L sulfuric acid TS to make 1000 mL.

Papaverine hydrochloride $C_{20}H_{21}NO_4 \cdot HCl$ [Same as the name sake monograph]

Paraffin [Same as the namesake monograph in Part II].

Paraffin, liquid [Same as the monograph in Part II Light Liquid Paraffin].

Parahydoroxybenzoic acid $C_7H_6O_3$, White crystals.
Melting point between 212 and 216 °C

Content not less than 98.0 %.
Assay Weigh accurately about 0.7 g of 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 determination, and make any necessary correction.

EachmL of 0.5 mol/L sodium hydroxide VS
= 69.06 mg of $C_7H_6O_3$

Peanut oil [Same as the namesake monographin Part II].

Pentane $CH_3(CH_2)_3CH_3$, Clear and colorless liquid.
Specific gruvity d_{20}^{20} between 0.620 and 0.650
Distilling range between 35.5 and 37 °C, less than 98 vol %.

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 diethyl ether.
Loss on drying not more than 7 % (0.5 g, 105 °C, constant mass).
Residue on ignition not more than 15 % (0.5 g).
Degree of digestion Dissolve 1 g of casein peptone in 10 mL of water, and perform the followin g test using this solution as the test solution: (1) Overlay 1 mL of the test solution with 0.5 mL of a mixture of 1 mL of acetic acid (100) and 10 mL of dilute ethanol: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results. (2) Mix 1mL 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 red-purple color is produced.
Nitrogen content not less than 10 % (105 °C, constant mass, after drying according to the Nitrogen Determination).

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: 70-72 %].

Perchloric acid TS Add 8.5mL of per chloric acid to water, and make 100mL.

Perchloric acid-dehydrated ethanol TS See perchloric acid-ethanol (99.5) TS.

Perchloric acid-ethanol (99.5) TS Add cautiously 25.5 mL of perchloric acid to 50 mL of ethanol (99.5), cool, and add ethanol (99.5) to make 100mL (3 mol/L).

Peroxidase-labelded bradykinin A solution of horseradish origin poeroxidase-bindig bradykinin in gelatin-phosphate buffer solution, pH 7.0. A colorless to light brown clear solution.

Peroxidase-labelded 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 8mL, and lyophilize. Dissolve this in 8 mL of water. Prepare before use.

Petrolatum [Same as the monograph in part II, Yellow Petrolatum or White Petrolatum]

Phenacetin $C_{10}H_{13}NO_2$ [Same as the namesake monograph].

o-Phenanthroline See 1,10-phenanthroline monohydrate.

o-Phenanthroline hydrochloride See 1,10-phenanthrolium chloride monohydrate.

1,10-Phenanthroline monohydrate $C_{12}H_8N_2 \cdot H_2O$ [Special class].

o-Phenanthroline TS See 1,10-phenanthroline TS.

Phenethylamine hydrochloride $C_6H_5CH_2NH_2 \cdot HCl$, White crystals or crystlline powder.
Melting point between 220 and 225 °C

Phenobarbital sodium $C_{12}H_{11}N_2NaO_3$ [Same as the namesake monograph].

Phenol C_6H_5OH [Special class].

Phenolphthalein $C_{20}H_{14}O_4$ [Special class].

Phenolphthalein-thymol blue TS Solution A: Dissolve 0.1 g of phenolphthalein in diluted ethanol (99.5) (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, add water to make 100 mL. Mix 2 volumes of solution A and 3 volumes of solution B before use.

Phenolphthalein TS Dissolve 1 g of phenolphthaleinin 100mL of ethanol (95).

Phenol red $C_{19}H_{14}O_5S$ [Special class]

Phenol red TS Dissolve 0.1 g of phenol red in 100mL of ethanol (95), and filter, if necessary.

Phenol red TS, dilute To 235 mL of a solution of ammonium nitrate (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 water to make 200 mL. 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.

Phenylalanine $C_9H_{11}NO_2$ [Same as the monograph L-Phenylalanine].

Phenyl benzoate $C_6H_5COOC_6H_5$ White crystals or crystalline powder, having a slight, characteristic odor.
Melting point between 68 and 70 °C
Purity Clarity of solution Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol: the solution is clear.

o-Phenylenediamine dihydrochloride $H_2NC_6H_4NH_2 \cdot 2HCl$ White to pale yellow or pale red crystals or crystalline powder.
Clarity A solution (1 in 20) is clear.
Content not less than 98.0 %.
Assay Weigh accurately about 0.15 g of o-Phenylenediamine dihydrochloride, dissolve in 50 mL of water, and titarate with 0.1 mol/L sodium hydroxide VS(potentiometric titration).

Phenylfluorone $C_{19}H_{12}O_5$[Special class].

Phenylfluorone-ethanol TS Dissolve 0.050 g of phenyl-fluorone in 10 mL of a mixture of ethanol (95) and diluted hydrochloric acid (1 in 3), and add thanol (95) to make exactly 500 mL.

D-Phenylglycine $C_8H_9NO_2$ White, crystals or crystalline powder. Slightly soluble in water.
Loss on drying : not more than 0.5 % (1 g, 105 °C, 3 hours).
Content : not less than 98.5 %.
Assay. Weigh accurately about 0.3 g of D-phenylglycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid VS
= 15.12 mg of $C_8H_9NO_2$

Phenylhydrazine $C_6H_5NHNH_2$ [Special class].

Phenylhydrazine hydrochloride See phenylhydrazinium chloride.

Phenylhydrazine hydrochloride TS See phenylhydra-zinium chloride TS.

Phenylhydrazinium chloride $C_6H_5NHNH_2 \cdot HCl$ [Special class].

Phenylhydrazinium chloride TS Dissolve 0.065 g of phenylhydrazinium chloride, recrystallized from dilute ethanol, in 100 mL of a solution previously prepared by adding cautiously 170 mL of sulfuric acid to 80 mL of water.

1-phenyl-3-methyl-5-pyrazolone See 3-methyl-1-phenyl-5-pyrazolone.

Phloroglucin $C_6H_3(OH)_3 \cdot 2H_2O$ [Special class].

Phosphatase TS, Alkaline Dissolve 95±5 mg of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

Phosphatase, Alkaline [first class]
Obtained from bovine small intestine. A white to grayish white or yellowish brown, lyophilized powder. It contains about 1 Units per mg, not containing any saline. One unit indicates an amount of the enzyme which produces 1 mmol of 4-nitrophenol at 37 °C and pH 9.8 in 1 minute from 4-nitrophenyl phosphate used as the substrate.

Phosphate buffer solution for pancreatin Dissolve 3.3 g of disodium hydrogen phosphate, 1.4 g of potassium dihydrogen phosphate and 0.33 g of sodium chloride in water to make 100 mL.

0.02 mol/L 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).

0.1mol/L Phosphate buffer solution, pH 4.5 Dissolve 13.61 g of sodium dihydrogen phosphate dihydrate in 750 mL of water, and adjust the pH to 4.5 with potassium hydroxide TS, and add water to make 100 mL.

Phosphate buffer solution, pH 6.0 Dissolve 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate in 750 mL of water, adjust the pH to 6.0 with sodium hydroxide TS or diluted phosphoric acid (1 in 15), and add water to make 1000 mL.

Phosphate buffer solution, pH 12 To 5.44 g of disodium hydrogen phosphate, add 36.5 mL of sodium hydroxide TS and about 40 mL of water, dissolve by shaking well, and add water to make 100 mL

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 VS, and add water to make 200 mL.

Phosphate buffer solution, pH 6.8 Dissolve 3.40 g of potassium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in water to make 1000 mL.

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 VS, and add water to make 200 mL.

0.05 mol/L Phosphate buffer solution, 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 to pH 7.0 with phosphoric acid or potassium hydroxide TS.

0.1 mol/L Phosphate buffer solution, pH 7.0 Dissolve 17.9 g of disodium hydrogen phosphate 12-hydrate in water to make 500 mL (solution A). Dissolve 6.8 g of potassium dihydrogen phosphate in water to make 500 mL (solution B). To a volume of solution A, add solution B until the mixture is adjusted to pH 7.0 (about 2:1 by volume of solutions A and B).

Phosphate buffer solution, pH 7.2 Mix 50mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

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 VS, and add water to make 200 mL.

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 VS, and add water to make 200 mL.

0.1 mol/L Phosphate buffer solution, pH 8.0 Dissolve 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate in about 750mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL.

Phosphate buffer solution for microplate washing Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate 12-hydrate, 52.6 g of sodium chloride, 3.0 g of polysorvate 80 and 1.8 g of polyoxyethylene (40) octylphenyl ether in water to make 600mL. Dilute this solution 10 times with water before use.

Phosphate pH standard solution See pH Determination under the General Tests, Processes and Apparatus.

Phosphomolybdic acid See phosphomolybdic acid n-hydrate.

Phosphomolybdic acid n-hydrate $P_2O_5 24MoO_3 \cdot nH_2O$ [Special class].

Phosphomolybdic acid-tungstic acid TS (Phosphomolybdotungstic TS) Dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700mL of water. Add 100mL of hydrochloric acid and 50mL of phosphoric acid R. Heat the mixture under a reflux condenser in a glass apparatus for 10 hours. Add 150 g of lithium sulphate, 50mL of water and a few drops of bromine. Boil to remove the excess of bromine (15 minutes), allow to cool, dilute to 1000mL with water and filter. The solution is yellow in color. If it acquires a greenish tint, it is unsatisfactory for use but may be regenerated by boiling with a few drops of bromine. Care must be taken to remove the excess of bromine by boiling. Storage: at 2 to 8 °C.

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 1000mL of water, and add 2.7mL of phosphoric acid. If necessary, adjust to pH 2.3 with 2-aminoethanol.

Phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0 Dissolve 6.77mL of phosphoric acid, 5.72mL of acetic acid (100) and 6.18 g of boric acid in water to make 1000mL. Adjust the pH of this solution to 2.0 with 0.5 mol/L sodium hydroxide VS.

Phosphorus pentoxide See phosphorus (V) oxide. Phosphorus, red [First class].

Phosphotungstic acid See phosphotungstic acid n-hydrate.

Phosphotungstic acid n-hydrate $P_2O_5 \cdot 24WO_3 \cdot nH_2O$ [Special class].

Phosphotungstic acid TS Dissolve 1 g of phosphotungstic acid in water to make 100mL.

Phthalazine $C_8H_6N_2$ Yellowtotancrystals.

Phthalic acid $C_6H_8O_4$, Colorless or white crystalline powder. Soluble in methanol or ethanol (95), sparingly soluble in water, and practically insoluble in chloroform.
Melting point between 205 and 209 °C (with decomposition).
Content not less than 98 %.
Assay Weigh accurately about 2.8 g of phthalic acid, add exactly 50mL of 1 mol/L sodium hydroxide VS and 25mL 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 determination, and make any necessary correction.

EachmL of 1 mol/L sodium hydroxide VS
= 83.06 mg of $C_6H_8O_4$

Phthalic anhydride $C_8H_4O_3$ [Special class].

Picric acid See 2,4,6-trinitrophenol.

Picric acid-ethanol TS See 2,4,6-trinitrophenol-ethanol TS.

Picric acid TS See 2,4,6-trinitrophenol TS.

Picric acid TS, alkaline See 2,4,6-trinitrophenol TS, alkaline.

Piperidine hydrochloride $C_5H_{11}N.HC1$, White, crystalline powder. Dissolves in water or methanol. The pH of the aqueous solution (1 in 20) is between 3.0 and 5.0.
Melting point between 240 and 245 °C
Purity Clarity and color of solution Dissolve 1.0 g of piperidine hydrochloride in 20mL of water: the solution is clear and colorless.
Residue on ignition more than 0.10 % (1 g).
Content not less than 99.0 %.
Assay Dissolve about 0.25 g of piperidine hydrochloride, accurately weighed, in 50mL of water, add 5mL of diluted nitric acid (1 in 3), and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L silver nitrate VS
= 12.161 mg of $C_5H_{11}N.HC1$

Polyoxyethylene hydrogenated castor oil 60 A nonionic surfactant prepared by addition of polymerization of ethylene oxide to hydrogenated castor oil. Average molar number of ethylene oxide added is about 60. A white or pale yellow petrolatum-like or waxy substance, having a faint, characteristic odor and a slight, bitter taste. Very soluble in ethyl acetate or chloroform, freely soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.
Identification (1) To 0.5 g of polyoxyethylene hydrogenated castor oil 60, add 10mL of water and 5mL of ammonium thiocyanate-cobaltous nitrate TS, and shake thoroughly., add 5mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer. (2) To 0.2 g of polyoxyethylene hydrogenated castor oil 60, add 0.5 g of potassium bisulfate, and heat: an acrolein-like, irritating odor is perceptible. (3) To 0.5 g of polyoxyethylene hydrogenated castor oil 60, add 10mL of water, shake, and add 5 drops of bromine TS: the color of the test solution does not disappear.
Congealing point 30-34 °C
pH To 1.0 g of polyoxyethylene hydrogenated castor oil 60, add 20mL of water, and dissolve by heating: the pH of the solution is between 3.6 and 6.0.
Acid value not more than 1.0.
Saponification value 41 -51
Hydroxyl value 39 - 49
Purity (1) *Clarityandcolorofsolution* Dissolve 1.0 g of polyoxyethylene hydrogenated castor oil 60 in 20mL of ethanol: the 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 2.0mL of standard lead solution (more than 20 ppm). (3) *Arsenic* Take 1.0g of polyoxyethylene hydrogenated castor oil 60, prepare the test solution according to Method 3, and perform the test (more than 2 ppm).
Water not more than 2.0 % (1 g).
Residue on ignition not more than 0.10 % (1 g).
Storage Preserve in tight containers.

Polysorbate 20 Chiefly consists of, addition polymer of sorbitan monolaurate and ethylene oxide. Pale yellow to yellow liquid, having a faint, characteristic odor.
Identification (1) To 0.5 g of polysorbate 20, add 10mL of water and 10mL 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 10mL of water, shake, 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 50mL of water, acidity with hydrochloric acid (methyl orange), and extract with two 30mL volumes of diethyl ether. Combine the diethyl ether layer, wash with 20mL volumes of water until the washings become neutral, and evaporate the diethyl ether on a water-bath: the acid value of the residue is between 275 and 285. Use 50mL of 0.5 mol/L potassium hydroxide-ethanol VS for saponi5cation.
Acid value not more than 4.0.
Saponification value 43 -55
Loss on drying not more than 3.0 % (5 g, 105 °C, 1 hour).
Residue on ignition Weigh accurately 3 g of polysorbate 20, heat gently at first, and ignite gradually (800 in 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., add the filtrate to it, evaporate to dryness, and ignite carefully until the carbonized substance does not remain. If any carbonized substance still remains, add 15mL of ethanol (95), 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: not more than 1.0 %.

Polysorbate 80 [Same as the namesake monograph in Part II].

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. It is tasteless. Practically insoluble in ethanol (95) or diethyl ether. To polyvinyl alcohol I, add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

Viscosity 25.0 -31.0 mm2/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95mL of water, allow to stand for 30 minutes, and heat to dissolve on a water-bath under a reflux condenser for 2 hours while stirring. 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 °C as directed in Method 1 under the Viscosity Determination.

pH The pH of a solution of polyvinyl alcohol I (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution To 1.0 g of polyvinyl alcohol I, add 20mL of water, disperse by well stirring, warm between 60 °C and 80 °C for 2 hours, and cool: the solution is colorless and clear.

Saponification value between 98.0 and 99.0 mol %. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered, conical flask, add 100mL of water, and dissolve by heating on a water-bath. After cooling, add exactly 25mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then, add exactly 30mL 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 determination 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.

$$\text{Saponification value (mol \%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a-b)f}{\text{amount(a)of the sample}}$$

a: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the test
b: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank test
f: Molarity 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. It is tasteless. Practically insoluble in ethanol (95) or diethyl ether. To polyvinyl alcohol II, add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

Viscosity between 4.6 and 5.4 mm2/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95mL of water, allow to stand for 30 minutes, and dissolve by stirring on a water-bath between 60 °C and 80 °C for 2 hours. 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 °C as directed in Method 1 under the Viscosity Determination.

pH The pH of a solution of polyvinyl alcohol II (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution To 1.0 g of polyvinyl alcohol II, add 20mL of water, disperse by well stirring, heat on a water-bath for 2 hours, and cool: the solution is clear and colorless.

Saponification value between 86.5 and 89.5 mol %. Weigh accurately about 2.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered, conical flask, add 100mL of water, and warm while stirring for 2 hours. After cooling, add exactly 25mL of 0.5 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then, add exactly 30mL 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 determination in the same manner, and make any necessary correction.

$$\text{Saponification value (mo1 \%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a-b)f}{\text{amount(a)of the sample}}$$

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test
f: Molarity factor of 0.5 mol/L sodium hydroxide VS

Polyvinyl alcohol TS Weigh exactly 0.50 g of polyvinyl alcohol, and add water to make exactly 100mL.

Potassium acetate $CH_3COO.K$ [Special class].

Potassium acetate TS Dissolve 10 g of potassium acetate in water to make 100mL (1 mol/L).

Potassium bromate $KBrO_3$ [Special class].

Potassium bromide KBr [Specialclass].

Polassium bromide for infrared spectrophotometry Crush homocrystals of potassium bromide or potassium bromide, collect a 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: any abnormal spectrum does not appear.

Potassium carbonate K_2CO_3 [Special class].

Potassium carbonate, anhydrous See potassium carbonate.

Potassium carbonate-sodium carbonate TS Dissolve 1.7 g of potassium carbonate and 1.3 g of anhydrous sodium carbonate in water to make 100mL.

Potassium chlorate $KClO_3$ [Special class].

Potassium chloride KCl [Special class].

Potassium chloride-hydrochloric acid buffer solution To 250mL of a solution of potassium chloride (3 in 20) add 53mL of 2 mol/L hydrochloric acid TS and water to make 1000mL.

Potassium chloride TS, acidic Dissolve 250 g of potassium chloride in water to make 1000mL, and add 8.5mL of hydrochloric acid.

0.2mol/L Potassium chloride TS Dissolve 14.9 g of potassium chloride in water to make 1000mL. Prepare before use.

Potassium chromate K_2CrO_4 [Special class].

Potassium chromate TS Dissolve 10 g of potassium chromate in water to make 100mL.

Potassium cyanide KCN [Special class].

Potassium cyanide TS Dissolve 1 g of potassium cyanide in water to make 10mL. Prepare before use.

Potassium dichromate $K_2Cr_2O_7$ [Special class].

Potassium dichromate (standard reagent) $K_2Cr_2O_7$ [Standard reagent for volumetric analysis].

Potassium dichromate-sulfuric acid TS Dissolve 0.5 g of potassium dichromate in diluted sulfuric acid (1 in 5) to make 100mL.

Potassium dichromate TS Dissolve 7.5 g of potassium dichromate in water to make 100mL.

Potassium dihydrogen phosphate KH_2PO_4 [Special class].

Potassium dihydrogen phosphate for pH determination KH_2PO_4 [for pH determination].

0.05mol/L Potassium dihydrogen phosphate, pH 3.0 Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid.

0.02 mol/L Potassium dihydrogen phosphate TS Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000mL.

0.05 mol/L Potassium dihydrogen phosphate TS Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000mL.

0.2 mol/L Potassium dihydrogen phosphate TS Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000mL.

0.2 mol/L Potsssium dihydrogen phosphate TS for buffer solution Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000mL.

0.05 mol/L Potassium dihydrogen phosphate TS, pH 4.7 Dissolve 6.80 g of potassium dihydrogen phosphate in 900mL of water, adjust the pH to exactly 4.7 with dilute sodium hydrochloride TS, and add water to make 1000mL.

Potassium disulfate $K_2S_2O_7$ [Potassium Disulfate, Special class].

Potassium ferricyanide See potassium hexacyanoferrate (III).

Potassium ferricyanide TS See potassium hexacyano-ferrate (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) trihydrate TS.

Potassium hexacyanoferrate (II) trihydrate $K_4Fe(CN)6·3H2O$ [Specialclass].

Potassium hexacyanoferrate (II) trihydrate TS Dissolve 1 g of potassium hexacyanoferrate (II) trihydrate in water to make 10mL (1/4 mol/L). Prepare before use.

Potassiumhexacyanoferrate (III) K3Fe(CN)6 [Special class].

Potassium hexacyanoferrate (III) TS Dissolve 1 g of potassium hexacyanoferrate (III) in water to make 10mL (1/3 mol/L). Prepare before use.

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 100mL. Preserve in light-resistant containers.

Potassium hexahydroxoantimanate (V) $K_2H_2Sb_2O_7·4H_2O$ [First class].

Potassium hexahydroxoantimanate (V) TS To 2 g of potassium hexahydroxoantimanate (V) add 100mL of water. Boil the solution for about 5 minutes, cool quickly, add 10mL of a solution of potassium hydroxide (3 in 20), allow to stand for 1 day, and filter.

Potassium hydrogen carbonate $KHCO_3$ [Special class].

Potassium hydrogen phthalate $C_6H_4(COOK)(COOH)$ [Special class].

Potassium hydrogen phthalate buffer solution, pH 3.5 Dilute 50mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97mL of 0.2 mol/L hydrochloric acid VS with water to make 200mL.

Potassium hydrogen phthalate buffer solution, pH 4.6 Dilute 50mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0mL of 0.2 mol/L sodium hydroxide VS with water to make 200mL.

Potassium hydrogen phthalate buffer solution, pH 5.6 Dilute 50mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7mL of 0.2 mol/L odium hydroxide VS with water to make 200mL.

Potassiam hydrogen phthalate for pH determination $C_6H_4(COOK)(COOH)$ [For pH determination].

Potassium hydrogen phthalate (standard reagent) $C_6H_4(COOK)(COOH)$ [Standard reagent for volumetric analysis].

0.2 mol/L Potassium hydrogen phthalate TS for buffer solution Dissolve 40.843 g of potassium hydrogen phthalate for pH determination in water to make 1000mL.

Potassium hydrogen sulfate $KHSO_4$ [Special class].

Potassium hydroxide KOH [Special class].

Potassium hydroxide TS Dissolve 6.5 g of potassium hydroxide in water to make 100mL (1 mol/L). Preserve in polyethylene bottles.

0.02 mol/L Potassium hydroxide TS Dilute 2mL of potassium hydroxide TS with water to make 100mL. Prepare before use.

0.05 mol/L Potassium hydroxide TS Dilute 5mL of potassium hydroxide TS with water to make 100mL. Prepare before use.

8 mol/L Potassium hydroxide TS Dissolve 52 g of potassium hydroxide in water to make 100mL. Preserve in polyethylene bottles.

Potassium hydroxide-ethanol TS Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100mL. Prepare before use.

0.1 mol/L Potassium hydroxide-ethanol TS To 1mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5mL. Prepare before use.

Potassium hydroxide-ethanol TS, dilute Dissolve 35 g of potassium hydroxide in 20mL of water, and add ethanol (95) to make 1000mL (0.5 mol/L). Preserve in tightly stoppered bottles.

0.1 mol/L Potassium hydroxide-methanol TS Dissolve 6.8 g of potassium hydroxide in 4mL of water, and add ethanol (95) to make 1000mL.

Potassium iodate KIO_3 [Special class].

Potassium iodate (standard reagent) KIO_3 [Standard reagent 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.
Storage Preserve in a glass-stoppered bottle, protected from light and moisture.

Potassium iodide KI [Special class].

Potassium lodide-starch paper Impregnate filter paper with freshly prepared potassium iodide-starch TS, and dry in a clean room. Store in a glass-stoppered bottle, protected from light and moisture.

Potassium lodide-starch TS Dissolve 0.5 g of potassium iodide in 100mL of **freshly** prepared starch TS. Prepare before use.

Potassieum iodide TS Dissolve 16.5 g of potassium iodide in water to make 100mL. Preserve in light-resistant containers. Prepare before use (1 mol/L).

Potassium iodide TS, concentrated Dissolve 30 g of potassium iodide in 70mL of water. Prepare before use.
Storage Preserve in light-resistant containers.

Potassium iodide-zinc sulfate TS Dissolve 5 g of potassium iodide, l0 g of zinc sulfate 7 hydrate, and 50 g of sodium chloride in water to make 200mL.

Potassium methanesulfonate CH_3SO_3K White crystals or crystalline powder.
Purity Clarity and color of solution: Dissolve 1.0 g of potassium ethanesulfonate in 20mL of water: the solution is transparent and colorless.
Content: not less than 98.0 %.
Assay Dissolve about 0.1 g of potassium methanesulfonate, accurately weighed, in 10mL of acetic acid (100), add 20mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiom-

etric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.42 mg of CH_3SO_3K

Potassium naphthoquinone sulfate See potassium 1,2-naphthoquinone-sulfonate.

Potassium 1,2-naphthoquinone-4-sulfonate $C_{10}H_5O_2SO_3K$ [β-Naphthoquinone-4-sulfonicAcid Potassium Salt, Special class].

Potassium 1,2-naphthoquiaone-4-sulfate TS Dissolve 0.5 g of potassium 1,2-naphthoquinone-sulfonate in water to make 100mL. Prepare before use.

Potassium nitrate KNO_3 [Special class].

Potassium nitrite KNO_2 A white to pale yellow crystalline powder. It is deliquescent.
Identification. (1) Dissolve 1 g of potassium nitrite in 20mL of water, and use this as the sample solution. To 5mL of the sample solution add 1mL of sulfuric acid: a yellowish brown gas is evolved.
(2) The sample solution obtained in (1) responds to the Qualitative Tests (1) for potassium salt.

Potassium perchlorate $KClO_4$ [Special class].

Potassium periodate KIO_4 [Special class].

Potassium periodate TS To 2.8 g of potassium periodate add 200mL of water, dissolve by adding dropwise 20mL of sulfuric acid under shaking, cool, and add water to make 1000mL.

Potassium permanganate $KMnO_4$ [Special class].

Potassium permanganate TS Dissolve 3.3 g of potassium permanganate in water to make 1000mL (0.02 mol/L).

Potassiam permanganate TS acidic To 100mL of potassium permanganate TS add 0.3mL of sulfuric acid.

Potassium persulfate See potassium peroxodisulfate.

Potassium pyroantimonate See potassium hexahydroxoantimonate (V).

Potassium pyroantimonste TS See potassium hexahydroxoantimonate (V) TS.

Potassium pyrophosphate $K_4O_7P_2$ White, crystalline powder, very soluble in water.
Melting point 1109 °C.

Potassium pyrosulfate See potassium disulfate.

Potassium sodium tartarate See potassium sodium tartarate tetrahydrate.

Potassium sodium tartarate tetrahydrate $KNaC_4H_4O_6$ [(+)-Potassium sodium tartratete trahydrate, Special class].

Potassium sulfate K_2SO_4 [Special class].

Potassium sulfate TS Dissolve 1 g of potassium sulfate in water to make 100mL.

Potassium thiocyanate KSCN [Special class].

Potassium thiocyanate TS Dissolve 1 g of potassium thiocyanate in water to make 10mL.

Potato starch TS Prepare as directed under starch TS with 1 g of potato starch.

Potato starch TS for amylolytic activity test Dry about 1 g of potato starch, accurately weighed, at 105 °C for 2 hours, and measure the loss. Weigh accurately an amount of potato starch, equivalent to 1.000 g on the dried basis, place into a conical flask, add 20mL of water, and make it pasty by gradually adding 5mL of a solution of sodium hydroxide (2 in 25) while shaking well Heat in a water bath for 3 minutes while shaking, add 25mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid TS, add 10mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0, and add water to make exactly 100mL. Prepare before use.

Prednisolone $C_{21}H_{28}O_5$ [Same as the name sake monograph].

Prednisone $C_{21}H_{26}O_5$ White, crystalline powder. Slightly soluble in methanol, in ethanol (95) and in chloroform, and very slightly soluble in water.
Specific Optical Rotation $[\alpha]_D^{20}$ between +167 and +175 (after drying, 0.1 g, 1,4-dioxane, 10mL, 100 mm).
Loss on drying not more than 1.0 % (1 g, 105 °C, 3 hours).
Content between 96.0 and 104.0 %.
Assay Weigh accurately about 0.02 g of prednisone, and dissolve in methanol to make exactly 100mL. Pipet 5mL of this solution, dilute with methanol to make exactly 100mL. Perform the test with this solution as directed under the Ultraviolet-visible Spectrophotometry, and read the absorbance, A , at the wavelength of maximum absorption at about 238 nm.

$$\text{Amount (mg) of } C_{21}H_{26}O_5 = \frac{A}{440} \leftarrow 20000$$

Procainamide hydrochloride $C_{13}H_{21}N_3O.HCl$ [Same as the name sake monograph].

Procaine hydrochloride $C_{13}H_{20}N_2O_2HC1$ [Same as the namesake monograph].

Procaterol hydrochloride $C_{16}H_{22}N_2O_3 \cdot HCl \cdot H_2O$ [Same as the namesake monograph].

Progesterone $C_{21}H_3O_2$ [Sameasthenamesakemonograph].

n-Propanol See 1-propanol.

1-Propanol $CH_3CH_2CH_2OH$ [Special class].

2-Propanol $(CH_3)_2CHOH$ [Special class].

Propantheline bromide $C_{23}H_{30}BrNO_3$[Same as the namesake monograph]

2-Propanol for liquid chromatography $(CH_3)_2CHOH$ Clear, colorless and volatile liquid, having a characteristic odor. Miscible with water, ethanol (95) or diethyl ether. Boiling point: about 82 °C.

Refractive index n_D^{20} between 1.376 and 1.378.

Specific gravity d_{20}^{20} between 0.785 and 0.788.

Purity (1) *Ultraviolet* a bsorbing substances Perform the test with 2-propanol for liquid chromatography as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbance at 230 nm is not more than 0.2; at 250 am, not more than 0.03; and between 280 nm and 400 nm, not more than 0.01. (2) *Peroxide* Mix 100mL of water and 25mL of dilute sulfuric acid, and add 25mL of a solution of potassium iodide (1 in 10). Add this solution to 20 g of 2-propanol for liquid chromatography. Stopper tightly, shake, allow to stand for 15 minute in a dark place, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 1mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005 %).

Propionic acid CH_3CH_2COOH Colorlessl iquid.

Purity Clarity and color of solution Dissolve 1 g of propionic acid in 20mL of ethanol (95): the solution is clear and colorless.

Specific gravity n_D^{20} between 0.998 and 1.004.

Distilling range between 139 and 143 °C, not less than 95 vol %.

Propylamine, iso $(CH_3)_2CHNH_2$A colorless liquid, having a characteristic, amine-like odor. Miscible with water, with ethanol (95) and with diethyl ether.

Refractive index n_D^{20} between 1.374 and 1.376.

Specific gravity d_{20}^{20} between 0.685 and 0.690.

Distilling range between 31 and 33 °C, not less than 95 vol %.

Propyl benzoate $C_6H_5COOC_3H_7$ A clear, colorless liquid, having a characteristic odor.

Refractive index n_D^{20} between 1.498 and 1.503.

Specific gravity d_{20}^{20} between 1.022 and 1.027.

Propylene glycol $CH_3CH(OH)CH_2OH$ [Special class].

Propylether, iso $(CH_3)_2CHOCH(CH_3)_2$ A clear, colorless liquid, having a characteristic odor. not miscible with water.

Refractive index n_D^{20} between 1.368 and 1.369

Specific gravity d_{20}^{20} between 0.723 and 0.725

Propyl parahydroxybenzoate $HOC_6H_4COOCH_2$ CH_2CH_3 [Same as the name sake monograph In Part II].

Prostaglandin A1 $C_{20}H_{32}O_4$ White crystals or crystalline powder. Very soluble in ethanol (95) on ethyl acetate, and very slightly soluble in water.

Purity Related substances Dissolve 5 mg of prostaglandin AD in 10mL of ethanol (95), and use this solution as the test solution. Pipet 3mL of the test solution, add ethanol (95) to make exactly 100mL, 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 operating 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 A1 from the test solution is not larger than the peak area of prostaglandin A1 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 lprostadil Alfadex.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of prostaglandin A1obtainedfrom10 μL of the standard solution composes 5 to 10 % of the full scale.

Time span of measurement: About twice as long as the retention time of prostaglandin A1 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 [Same as the namesake monograph in Part II].

Purified water for ammonium limit test To 1500mL of purified, add cautiously 4.5mL of sulfuric acid, distil using a hard glass distiller, discard the first

distillate, and use the remaining distillate as ammonium-free purified water.
Purity Mix 40mL of purified water for ammonium limit test with 6.0mL of phenol-sodium pentacyanonitrosylfer-rate (III) TS. Add 4.0mL of sodium hypochlorite-sodium hydroxide TS, mix, and allow to stand for 60 minutes. Perform the test with this solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: Absorbance at the wavelength of 640 nm is not more than 0.010.

Pyrazole $C_3H_4N_2$ White to pale yellow crystals or crystalline powder.
Melting point between 67 and 71 °C

Pyridine C_5H_5N [Speial class].

Pyridine, anhydrous C_5H_5N To 100mL of pyridine add 10 g of sodium hydroxide, and allow to stand for 24 hours. Decant the supernatant liquid, and distill.

Pyridine-acetic acid TS Dilute 20mL of pyridine with sufficient diluted acetic acid (100) (1 in 25) to make 100mL. Prepare before use.

Pyridine for Karl Fischer method See Water Determination

Pyridine-pyrazolone TS Dissolve, with thorough shaking, 0.1 g of 3-methyl-1-phenyl-5-pyrazolone in 100mL of water by heating between 65 °C and 70 °C, and cool below 30 °C. Mix this solution with a solution prepared by dissolving 0.02 g of bis-(1-phenyl-3-methyl-5-pyrazolone) in 20mL of pyridine. Prepare before use.

Pyridoxine hydrochloride $C_8H_{11}NO_3.HCl$ [Same as the namesake monograph].

Pyrogallol $C_6H_3(OH)_3$ [Special class].

L-Pyroglulamylglycyl-L-arginine-p-nitroanilide TS Dissolve 0.025 g of L-pyroglutamylglycyl-L-arginine-p-nitroanilide hydrochloride and 0.04 g of D-Mannitol in 2 to 3mL of water, lyophilyze, and dissolve in 16.7mL of water. To 1 volume of this solution, add 9 volumes of water before use.

L-Pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride $C_{19}H_{26}N_8O_6.HCl$ White to light powder. Freely soluble in water, methanol or glacial acetic acid (100).
Absorbance $E_{1\,cm}^{1\%}$ (316 nm) between 242 and 268 (2 mg, water, 100mL).
Specific Optical Rotation $[\alpha]_D^{20}$ between -51° and -56° [0.1 g, diluted acetic acid (100) (1 in 2), 10mL, 100 mm].
Purity Related substances Dissolve 0.05 g of L-pyroglutamylglycyl-L-arginine-p-nitroaniline hydrochloride in 10mL of methanol, and use this solution as the test solution. Pipet 1mL of the test solution, add methanol to make exactly 50mL, and use this solution μL as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 L each of the test solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of n-butanol, water, pyridine and acetic acid (100) (15 : 12 : 10 : 3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Pyrole C_4H_5N A clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) or diethyl ether, and practically insoluble in water.
Specific gravity d_{20}^{20} between 0.965 and 0.975.

Quinhydrone $C_6H_4(OH)_2.C_6H_4O_2$ [Specialclass]. Green crystals or crystalline powder.
Melting point 169 ~ 172 ℃

Quinoline C_9H_7N [Special class].

Quinoline TS Mix 50mL of quinoline with 300mL of diluted hydrochloric acid (1 in 6), previously heated, cool, and filter, if necessary.

8-Quinolinol C9H7NO[Special class].

Raney nickel catalyst Grayish black powder. An alloy containing 40 to 50 % of nickel and 50 to 60 % of aluminum.

Red litmus paper See litmus paper, red.

Reinecke salt See reinecke salt monohydrate.

Reinecke salt monohydrate $NH_4[Cr(NH_3)_2(SCN)_4]\cdot H_2O$ Dark red crystals or crystalline powder.
Identification Determine the infrared absorption spectrum of Reinecke salt monohydrate as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3310 cm^{-1}, 2130 cm^{-1}, 1633 cm^{-1}, 1400 cm^{-1}, 1261 cm^{-1} and 711 cm^{-1}.

Reinecke salt TS To 20mL of water, add 0.5 g of Reinecke salt monohydrate, shake frequently for 1 hour, then filter. Use within 48 hours.

Resazurin $C_{12}H_6NNaO_4$ A brownish purple powder. It dissolves in water and the solution is purple in color.
Residue on ignition : not less than 28.5 % (1 g).

Resorcin See resorcinol.

Resorcinol $C_6H_4(OH)_2$ [Resorcinol, Special class].

Resorcinol sulfuric acid TS Dissolve 0.1 g of resorcinolin 10mL of diluted sulfuric acid (1 in 10).

Resorcinol TS Dissolve 0.1 g of resorcinol in 10mL of hydrochloric acid. Prepare before use.

Resorcin sulfuric acid TS See resorcinol sulfuric acid TS.

Resorcin TS See resorcinol TS.

Rhodamine B (Tetraethylrhodamine) $C_{28}H_{31}ClN_2O_3$ Green crystals or a reddish-violet powder. Very soluble in water, yielding a bluish-red solution that is strongly fluorescent when dilute. Very soluble in alcohol; slightly soluble in dilute acids and in alkali solutions. In strong acid solution, it forms a pink complex with antimony that is soluble in isopropyl ether.
Clarity of solution Its solution (1 in 200) is complete and clear.
Residue on ignition (Reagent test) Ignite 1 g with 1mL of sulfuric acid: the residue weighs not more than 2 mg (0.2 %).

Riboflavin $C_{17}H_{20}N_4O_6$ [Same as the namesake monograph].

Riboflavin sodium phosphate $C_{17}H_{20}N_4NaO_9P$ [Same as the namesake monograph]

Rose bengal $C_{20}H_2Cl_4I_4Na_2O_5$ [Special class]

Rose bengal TS Dissolve 1 g of Rose Bengal in water to make 100mL

Saccharated pepsin [Same as the namesake monograph in Part II].

Saffron [Same as the name sake monograph in Part II].

Salicylaldazine $C_{14}H_{12}N_2O_2$ Dissolve 0.30 g of hydrazinium sulfate in 5mL of water. To this solution, add 1mL of acetic acid (100) and 2mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well, and allow to stand until a yellow precipitate is produced. Extract with two 15mL volumes of dichloromethane, to the combined dichloromethane extracts add 5 g of anhydrous sodium sulfate, shake, decant or filter, and evaporate the dichloromethane in the supernatant liquid or filtrate. Dissolve the residue in a warmed mixture of toluene and methanol (6:4), and cool. Filter the crystals produced, and dry in a desiccator (in vacuum, silica gel) for 24 hours. It is a yellow, crystalline powder.
Melting point between 213 and 219 °C.
Purify Dissolve 0.09 g of salicylaldazine in toluene to make exactly 100mL. Pipet 1mL 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 HONCHO [Special class].

Salicylic acid HOC_6H_4COOH [Special class].

Scopolamine hydrobromide hydrate $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ [Same as the name sake monograph].

Sea sand [Special class].

Selenious acid H_2SeO_3 [Special class]. Colorless or white crystals. It is hygroscopic.
Identification. (1) Dissolve 0.2 g of selenious acid in 20mL of water, and use this as the sample solution. To 10mL of the sample solution add 2mL of tin (II) chloride TS: a red precipitate is produced.
(2) To 10mL of the sample solution obtained in (1) add 1mL of diluted hydrochloric acid (2 in 3) and 1mL of potassium iodide TS: a brown color is produced. Preserve in a light-resistant tight container.

Selenious acid-sulfuric acid TS Dissolve 0.05 g of selenious acid in 10mL of sulfuric acid.

Semicarbazide acetate TS Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30mL 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 100mL. Preserve in a cold place. Do not use the solution showing a yellow color.

L-Serine $C_3H_7NO_3$ [Special class]

Sesame oil [Same as the namesake monograph in Part II].

Silica gel An amorphous, partly hydrated silicic acid occurring in glassy granules of various sizes. When used as a desiccant, it is frequently coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated by being heated at 110 °C until the gel assumes the original color.
Residue on ignition not more than 6 % (2 g, 950 ± 50 °C).
Water absorption not less than 31 %. Weigh accurately about 10 g of silica gel, and allow to stand for 24 hours in a closed container in which the atmosphere is maintained at 80 % relative humidity with sulfuric acid having a specific gravity of 1.19. Weigh again, and calculate the increase in mass.

Silicone oil A colorless, clear liquid,having no odor.
Viscosity between 50 and 100 mm2/s

Silicone resin A light gray, half-clear, viscous liquid or a pasty material. It is almost odorless.
Viscosity and refractive index Place 15 g of silicone resin in a Soxhlet extractor, then extract with 150mL of carbon tetrachloride for 3 hours. The viscosity of the residual liquid, obtained by evaporating carbon tetrachloride from the extract on a water bath, is 100 to 1100 centistokes (25 °C). Its refractive index is 1.400 to 1.410 (25 °C).
Specific gravity between 0.98 and 1.02
Loss on drying between 0.45 and 2.25 g with the extracted residue obtained in the Viscosity and refractive index (100 °C, 1 hour).

Silicotungstic acid 26-hydrate $SiO_2 \cdot 12WO_3 \cdot 26H_2O$ White to slightly yellowish, crystals. Deliquescent. Verysoluble in water and in ethanol(95)
Loss on ignition 14.1 % (2g, dry at 110 °C for 2 hours then 700-750 °C, constant mass).
Clarity and color of solution a solution (1 in 20) is clear and colorless

Silver nitrate $AgNO_3$[Special class].

Silver nitrate-ammonia TS Dissolve 1 g of silver nitrate in 20mL of water, and add ammonia TS dropwise with stirring until the precipitate is almost entirely dissolved.
Storage Preserve in tight, light-resistant containers.

Silver nitrate TS Dissolve 17.5 g of silver nitrate in water to make 1000mL (0.1 mol/L). Preserve in light-resistant containers.

Silver diethyldithiocarbamate See Silver *N,N*-diethyl dithiocarbamate

Silver *N,N*-diethyldithiocarbamate $C_5H_{10}AgNS_2$.

Soda lime [First class].

Sodium Na [Special class].

Sodium acetate See sodium acetate trihydrate.

Sodium acetate-acetone TS Dissolve 8.15 g of sodium acetate trihydrate and 42 g of sodium chloride in 100mL of water, and add 68mL of 0.1 mol/L hydrochloric acid VS, 150mL of acetone and water to make 500mL.

Sodium acetate, anhydrous CH_3COONa [Specia lclass].

Sodium acetate buffer solution Dissolve 86.5 g sodium hydroxide and 10.3 g of sodium acetate trihydrate and water to make 1000mL.

Sodium acetate trihydrate $CH_3COONa \cdot 3H_2O$ [Special class].

Sodium acetate Dissolve 13.6 g of sodium acetate tri-hydrate in water to make 100mL (1 mol/L).

Sodium benzoate $NaC_6H_5CO_2$ [Special class].

Sodium biphenyl $C_{12}H_9Na$ Supplied as a solution(10 percent to 30 percent, w/w) in a mixture of dimethoxyethane and toluene or xylene. The solution is a viscous, dark green liquid. The solution deteriorates at a rate of about 10 % per month. Use only freshlu prepared soluition.
Activity Place 20mL of dry toluene in a titration flask equipped with a magnetic stirring bar and a stopper having a hole through which the delivery tip of a weight buret may be inserted. Add a quantity of sodium biphenyl sufficient to produce a blue color in the mixture, and titrate with amyl alcohol, contained in a weight buret, to the disappearance of the blue color. (Disregard the amounts of sodium biphenyl and amyl alcohol used in this adjustment.) Weigh accurately the weight buret containing the amyl alcohol. Transfer the contents of a vial of well-mixed test specimen to the titration flask, and titrate quickly with the amyl alcohol to the disappearance of the blue color. Weigh the buret to determine the weight of amyl alcohol consumed, and calculate the activity, in mEq/vial, by the formula: Result = $11.25W$ in which W is the weight of amyl alcohol consumed.
Not less than 10 % activity is found.
Iodine content Add 10mL to 5mL of toluene contained in a 125mL separator fitted with a suitable inert plastic stopcock, and shake vigorously for 2 min. Extract gently with three 10mL portions of dilute phosphoric acid (1 in 3), combining the lower phases in a 125mL iodine flask. Add sodium hypochlorite TS, dropwise, to the combined extracts until the solution turns brown, then add 0.5mL in excess. Shake intermittently for 3 min, add 5mL of freshly prepared, saturated phenol solution, mix, and allow to stand for 1 min, accurately timed. Add 1 g of potassium iodide, shake for 30 s, add 3mL of starch TS, and titrate with 0.1 N sodium thiosulfate VS: NMT 0.1mL of 0.1 N sodium thiosulfate is consumed.

Sodium bismuthate See bismuth sodium trioxide

Sodium bisulfite See sodium hydrogen sulfite.

Sodium bisulfte TS See sodium hydrogen sulfite TS.

Sodium borate decahydrate See sodium tetraborate decahydrate.

Sodium borate for pH determination See sodium tetraborate for pH determination.

Sodium borohydride $NaBH_4$ White to grayish white, crystals, powder or masses, Freely soluble in water.
Content : not less than 95 %.
Assay Weigh accurately 0.25 g of sodium borohydride, dissolve in 20mL of diluted sodium hydroxide TS (30 in 100), and add water to make exactly 500mL. Pipet 20mL of this solution, put in a glass stoppered iodine flask, and cool in ice. Add exactly 40mL of iodine TS, allow to stand at a dark place for 10 mimutes. Add exactly 10mL of diluted sulfuric acid (2 in 12), and titrate with 0.1 mol/L sodium thiosulfate VS(back titration) (indicator: starch solution). Perform a blank determination in the same manner, and make any necessary correction.

EachmL 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, anhydrous Na_2CO_3 [Sodiumcarbonate, Special class].

Sodium carbonate decahydrate $Na_2CO_3 \cdot 10H_2O$ [Special class].

Sodium carbonate for pH determination Na_2CO_3 [for pH determination].

Sodium carbonate (standard reagent) Na_2CO_3 [Standard reagent for volumetric analysis].

Sodium carbonate TS Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100mL (1 mol/L).

Sodium carbonate TS, 0.55 mol/L Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100mL.

Sodium chloride (standard reagent) NaCl [Standard reagent for volumetric analysis].

Sodium chloride TS Dissolve 10 g of sodium chloride in water to make 100mL.

Sodium chloride TS, 0.1 mol/L Dissolve 6 g of sodium chloride in water to make 1000mL.

Sodium citrate See sodium citrate hydrate.

Sodium citrate hydrate $C_6H_5Na_3O_7 \cdot 2H_2O$ [Special class, or same as the namesake monograph]

Sodium cyanide NaCN [Special class].

Sodium cyanide TS Dissolve 9 g of sodium cyanide in about 80mL of water, adjust the pH, with cautious stirring, to 5.5 with acetic acid (100), and add water to make 100mL. Prepare in a hood cautiously before use.

Sodium 1-decanesulfonate $C_{10}H_{21}NaO_3S$ A white powder.
Purity Clarity and color of solution. Dissolve 1.0 g in 20mL of water: the solution is clear and colorless.
Loss on drying not more than 3.0 % (1 g, 105 °C, 3 hours).
Content : not less than 98.0 %.
Assay. Weigh accurately about 0.45 g of sodium 1-decanesulfonate, dissolve in 50mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20mL of strongly acidic ionexchange resin (0.3 to 1.0 mm, H type) at a flow rate of about 4mL pe minute. Wash with 150mL of water at a flow rate of about 4mL per minute. Combine the washing and the elute, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $C_{10}H_{21}NaO_3S$

Sodium desoxycholate $C_{24}H_{39}NaO_4$ White, odorless, crystalline powder.
Identification Determine the infrared absorption spectrum of sodium desoxycholate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorptions at the wave numbers of about 3400 cm^{-1} 2940cm^{-1}, 1562cm^{-1}, and1408cm^{-1}
Purity Related substances Dissolve 0.10 g of sodium desoxycholate in 0mL of methanol, and use this solution as the test solution. Pipet 1mL of this solution, add methanol to make exactly 100mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. 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 concentrated sulfuric acid on the plate, and heat at 105 °C for 10 minutes: the spots other than the principal spot from the test solution are not more intense than the spot from the standard solution.

Sodium diethyldithiocarbamate See sodium *N,N*-diethyldithiocarbamate trihydrate.

Sodium N,N-diethyldithiocarbamate trihydrate $(C_2H_5)_2NCS_2Na.3H_2O$ [Special class]

2 mol/L Sodium dihydrogeaphosphate TS Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000mL.

Sodium dihydrogen phosphate dehydrate $NaH_2PO_4 \cdot 2H_2O$ [Special class].

0.05 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000mL.

0.1 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450mL of water, adjust to a pH of 5.8 exactly with sodium hydroxide TS, and add water to make 500mL.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 2.6 Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900mL of water, adjust the pH to exactly 2.6 and add water to make 1000mL.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 3.45 g of sodium dihydrogen phosphate dihydrate in 500mL of water (solution A). Dilute 2.45 g of phosphoric acid with water to make 500mL (solution B). To a volume of solution A, add solution B until the mixture is adjusted to pH 3.0.

0.1 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 15.60 g of sodium dihydrogen phosphate dihydrate in 900mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000mL.

Sodium dodecylbenzene sulfonate $C_{18}H_{29}SO_3Na$ A white crystalline powder or mass.
pH The pH of a solution of 0.5 g of sodium dodecylbenzene sulfonate in 50mL of freshly boiled and cooled water is between 5.0 and 7.0. Measure the pH at 25 °C passing nitrogen with stirring.
Loss on drying not more than 0.5 % (1 g, 105 °C, 2 hours).
Content not less than 99.0 %.
Assay Weigh accurately about 0.04 g of sodium dodecylbenzene sulfonate, previously dried, and perform the test as directed in (4) Sulfur in the Procedure of determination under the Oxygen Flask Combustion Method, using a mixture of 20mL of water and 2mL of strong hydrogen peroxide water as absorbing solution.

EachmL of 0.005 mol/L barium perchlorate VS
= 1.7424 mg of $C_{18}H_{29}SO_3Na$

Sodium fluoride NaF [Special class].

Sodium fluoride (standard reagent) NaF [Standard reagent for volumetric analysis].

Sodium fluoride TS Dissolve 0.5 g of sodium fluoride in 100mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.

Sodium hydrogen carbonate $NaHCO_3$ [Special class].

Sodium hydrogen carbonate for pH determination $NaHCO_3$ [sodium hydrogen carbonate, for pH determination].

Sodium hydrogen carbonate TS Dissolve 5.0 g of sodium hydrogen carbonate in water to make 100mL.

Sodium hydroxide NaOH [Special class].

Sodium hydroxide-dioxane TS Dissolve 0.80 g of sodium hydroxide in a mixture of 1,4-dioxane and water (3:1) to make 100mL.

Sodium hydroxide-methanol TS Dissolve by thorough shaking 4 g of sodium hydroxide in methanol to make 100mL. To the supernatant liquid obtained by centrifugation, add methanol to make 500mL. 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 1000mL.

Sodium hydroxide TS Dissolve 4.3 g of sodium hydroxide in water to make 100mL (1 mol/L). Preserve in polyethylene bottles.

Sodium hydroxide TS, dilute Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000mL. Prepare before use (0.1 mol/L).

0.1 mol/L Sodium hydroxide TS for bacterial endotoxins test Dissolve 4.3 g of sodium hydroxide in water for bacterial endotoxins test to make 1000mL.

Sodium hydroxide TS, 0.01 mol/L Dilute 10mL of sodium hydroxide TS with water to make 1000mL. Prepare before use.

Sodium hydroxide TS, 0.02 mol/L To 10mL of 0.2 mol/L sodium hydroxide TS, add water to make 100mL. Prepare before use.

Sodium hydroxide TS, 0.05 mol/L To 10mL of 0.5 mol/L sodium hydroxide TS, add water to make 100mL. Prepare before use.

Sodium hydroxide TS, 0.1 mol/L Dissolve 4.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000mL. Prepare before use.

Sodium hydroxide TS, 0.2 mol/L Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000mL. Prepare before use.

Sodium hydroxide TS, 0.5 mol/L Dissolve 22 g of sodium hydroxide in water to make 1000mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 1 mol/L Dissolve 4.3 g of sodium hydroxide in water to make 100mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 2 mol/L Dissolve 8.6 g of sodium hydroxide in water to make 100mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 4 mol/L Dissolve 168 g of sodium hydroxide in water to make 1000mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 5 mol/L Dissolve 220 g of sodium hydroxide in water to make 1000mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 8 mol/L Dissolve 336 g of sodium hydroxide in water to make 1000mL. Preserve in polyethylene bottles.

Sodium hydroxide TS, 10 mol/L Dissolve 440 g of sodium hydroxide in water to make 1000mL. Preserve in polyethylene bottles.

Sodium hypochlorite-sodium hydroxide TS To a volume of sodium hypochlorite TS for ammonium limit test, equivalent to 1.05 g of sodium hypochlorite (NaClO: 74.44), add 15 g of sodium hydroxide and water to make 1000mL. Prepare before use.

Sodium hypochlorite TS Prepare the solution by passing chlorine into sodium hydroxide TS while cooling with ice, so as to contain 5 % of sodium hypochlorite (NaClO: 74.44). Prepare before use.

Sodium hypochlorite TS for ammonium limit test A clear, colorless or pale green-yellow solution prepared by passing chlorine into sodium hydroxide or sodium carbonate solution, having the odor of chlorine.
Content not less than 4.2 w/v % as sodium hypochlorite (NaClO: 74.44).
Assay Pipet 10mL of sodium hypochlorite TS for ammonium limit test, and add water to make exactly 100mL. Transfer exactly 10mL of this solution to a glass-stoppered flask, add 90mL of water, then add 2 g of potassium iodide and 6mL of diluted acetic acid (1 in 2), stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

EachmL of 0.1 mol/L sodium thiosulfate VS
= 3.7221 mg of NaClO.

Sodium lauryl sulfate [Same as the namesake monograph in Part II]

Sodium metabisulfite See sodium disufite.

Sodium metabisulfiteTS See sodium disulfite TS.

Sodium, metallic Na [Sodium, Special class].

Sodium Methoxide CH_3ONa

Sodium molybdate See sodium molybdate dihydrate.

Sodium molybdate dihydrate $Na_2MoO_4.2H_2O$ [Special class].

Sodium 2-naphthalenesulfonate $C_{10}H_7NaO_3S$ Pale brown, crystals or powder.
Content : not less than 98.0 %.

Sodium naphthoquinone sulfonate TS Dissolve 0.25 g of sodium *p*-naphthoquinone sulfonate in methanol to make 100mL.

Sodium nitrate $NaNO_3$ [Special class]

Sodium nitrite $NaNO_2$ [Special class].

Sodium nitrite TS Dissolve 10 g of sodium nitrite in water to make 100mL. Prepare before use.

Sodium nitroprusside See sodium pentacyanonitrosylferrate (III) dihydrate.

Sodium nitroprusside TS See sodium pentacyanonitrosylferrate (III) TS.

Sodium oxalate (standard reagent) $Na_2C_2O_4$ [Standard reagent for volumetric analysis].

Sodium pentacyanoammine-ferroate Na_3 [Fe(CN)5-NH3]·nH2O[First class].

Sodium pentacyanonitrosylferrate(III)dehydrate $Na_2Fe(CN)_5(NO)\cdot 2H_2O$ [Special class].

Sodium pentacyanonitrosylferrate (III) TS Dissolve 1 g of sodium pentacyanonitrosylferrate (III) dihydrate in water to make 20mL. Prepare before use.

Sodium perchlorate See sodium perchlorate monohydrate.

Sodium periodate NaIO4 [Special class].

Sodium periodate TS Dissolve 60.0 g of sodium periodate in 120mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000mL. If the solution is not clear. filter this through a glass-filter. Keep in a light-resistant vessel.

Sodium peroxide Na_2O_2 [Special class].

Sodium phosphate See trisodium phosphate 12-hydrate.

Sodium phosphate TS Dissolve 5.68 g of disodium hydrogenophosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000mL.

Sodium p-phenol sulfonate $C_6H_5O_4NaS \cdot 2H_2O$ White to light yellow crystals or crystalline powder, having a specific odor.
Identification (1) To 10mL of a solution of sodium p-phenol sulfonate (1 in 10), add 1 drop of iron (III) chloride TS: a purple color develops. (2) Determine the absorption spectrum of a solution of sodium p-phenol sulfonate (1 in 5000) as directed under the Ultraviloletvisible Spectrophotometry: it exhibits maxima between 269 nm and 273 nm, and between 276 nm and 280 nm.
Purity Clarity and color of solution Dissolve 1.0 g of sodium p-phenol sulfonate in 25mL of water: the solution is clear and colorless.
Content not less than 90.0 %.
Assay Dissolve about 0.5 g of sodium p-phenol sulfonate, accurately weighed, in 50mL of water. Transfer the solution to a chromatographic column, prepared by pouring strongly acidic ion exchange resin (H type) for column chromatography (150 to 300 m in particle diameter) into a chromatographic tube, about 1 cm in inside diameter and about 30 cm in height, and allow to flow. Wash the chromatographic column with water until the washing is no longer acidic, combine the washings with the above effluent solution, and titrate with 0. 1 mol/L sodium hydroxide VS (indicator: 5 drops of bromocresol green-methyl red TS). Separately, dissolve 0.5 g of sodium p-phenol sulfonate, previously weighed accurately, in 50mL of water and titrate with 0.1 mol/L sodium hydroxide VS, and make any necessary correction.

EachmL of 0.1 mol/L sodium hydroxide VS
= 23.219 mg of $C_6H_5O_4NaS \cdot 2H_2O$

Sodium pyrosulfite $Na_2S_2O_5$ [Same as the namesake monograph].

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 VS to make 100mL.

Sodium selenite Na_2SeO_3 [Specialclass]A white crystalline powder.
Identification. (1) Dissolve 1 g of sodium selenite in 100mL of water, and use this as the sample solution. To 10mL of the sample solution add 2mL of tin (II) chloride TS: a red precipitate is produced.
(2) The sample solution obtained in (1) responds to the Qualitative Tests <1.09> (1) for sodium salt. Preserve in a light-resistant tight container.

Sodium p-styrenesulfonate $C_8H_7NaO_3S$ White crystals or crystalline powder. Freely soluble in water, slightly soluble in ethanol (99,5), and practically insoluble in diethyl ether. Recrystalize from diluted ethanol (1 in 2), and dry in vacuum.
Identification Determine the infrared absorption spectrum of sodium p-styrenesulfonate according to the potassium bromide disk method under the Infrared Spectrophotometry : it exhibit absorptions at the wave number of about $1236cm^{-1}$, $1192cm^{-1}$, $1136cm^{-1}$, $1052cm^{-1}$, $844cm^{-1}$ and $688cm^{-1}$.
Purity Perform the test with 10μL of a solution of sodium *p*-styrenesulfonate (1 in 1000) as directed in the Assay under Panipenem: any obstructive peaks for determination of panipene are not observed.

Sodium sulfate See Sodium sulfate decahydrate.

Sodium sulfate, anhydrous Na_2SO_4 [Special class].

Sodium sulfate decahydrate $Na_2SO_4 \cdot 10H_2O$ [Special class].

Sodium sulfide See sodium sulfide enneahydrate.

Sodium sulfide enneahydrate $Na_2S \cdot 9H_2O$ [Special class]

Sodium sulfide TS Dissolve 5 g of sodium sulfide enneahydrate in a mixture of 10mL of water and 30mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30mL of water and 90mL of glycerin, saturate a half volume of this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

Sodium sulfite See sodium sulfite heptahydrate.

Sodium sulfite, anhydrous Na_2SO_3 [Sodium sulfite, Special class].

Sodium sulfite heptahydrate $Na_2SO_3 \cdot 7H_2O$ [Special class].

Sodium tartrate See sodium tartrate dihydrate.

Sodium tartrate dihydrate $C_4H_4Na_2O_6 \cdot 2H_2O$ [Special class].

Sodium tetraborate decahydrate $Na_2B_4O_7 \cdot 10H_2O$ [Special class].

Sodium tetraborate for pH determination [for pH determination].

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 acd TS, and add water to make 1000mL.

Sodium thioglycolate $HSCH_2COONa$ [Specialclass]. Preserve in containers, protected from light and in a dark, cold place.

Sodium thiosulfate See sodium thiosulfate pentahydrate.

Sodiam thiosulfate pentahydrate $Na_2S_2O_3.5H_2O$ [Special class].

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 1000mL (0.1 mol/L).

Sodium 1-heptane sulfonate $C_7H_{15}NaO_3S$ White crystals or crystalline powder.
Purity Clarity and color of solution Dissolve 1.0 g of sodium 1-heptane sulfonate in 10mL of water: the solution is clear and colorless.
Loss on drying not more than 3.0 % (1 g, 105 °C, 3 hours).
Content not less than 99.0 %.
Assay Dissolve about 0.4 g of sodium 1-heptane sulfonate, previously dried and weighed accurately, in 50mL of water, transfer to a chromatographic column, prepared by packing a chromatographic tube, 9 mm in inside diameter and 160 mm in height with 10mL of strongly acidic ion exchange resin for column chromatography (425 to 600 μm in particle diameter, H type), and flow at a flow rate of about 4mL per minute. wash the column at the same flow rate with 150mL of water, combine the washings with the effluent solution, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the color of the solution changes from yellow to blue.

EachmL of 0.1 mol/L sodium hydroxide VS
= 20.225 mg of $C_7H_{15}NaO_3S$

Sodium 1-octanesulfonate $CH_3(CH_2)_7SO_3Na$ White crystals or powder.
Residue on ignition between 32.2 and 33.0 % (1.0 g).

Sodium 1-pentane sulfonate $C_5H_{11}NaO_3S$ White crystals or crystalline powder. Freely soluble in water, and practically insoluble in acetonitrile.
Purity Clarity and color of solution Dissolve 1.0 g of sodium 1-pentane sulfonate in 10mL of water: the solution is colorless and clear.
Water not more than 3.0 % (0.2 g).
Content not less than 99.0 percent, calculated on the anhydrous basis.
Assay Dissolve about 0.3 g of sodium 1-pentane sulfonate, accurately weighed, in 50mL of water. Transfer this solution to a chromatographic column, prepared by pouring 10mL of strongly acidic ion-exchange resin (H type) (424 to 600 μm in particle diameter) into a chromatographic tube, 9 mm in inside diameter and 160 mm in height, and elute at the rate of about 4mL per minute. Wash the chromatographic column with 50mL of water at the rate of about 4mL per minute, and wash again with 100mL of water in the same manner. Combine the washings with the eluate, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the yellow color of the solution changes to blue.

EachmL of 0.1 mol/L sodium hydroxide VS
= 17.420 mg of $C_5H_{11}NaO_3S$

Sodium β-napbthoquinone sulfonate $C_{10}H_5NaO_5S$ Yellow to orange-yellow crystals or crystalline powder. Soluble in water, and practically insoluble in ethanol (95).
Loss on drying not more than 2.0 % (1 g, in vacuum, 50 °C).
Residue on ignition Between 26.5 and 28.0 % (1 g, after drying).

Sodium tetraphenyl-borate $(C_6H_5)_4BNa$ [Special class].

Soluble starch See starch, soluble.

Soluble starch TS Triturate 1 g of soluble starch in 10mL of cooled water, pour gradually into 90mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

D- Sorbitol [Same as the namesake monograph].

Soybean peptone See peptone, soybean.

Soybean oil [Same as the namesake monograph in Part II].

Stannous chloride See tin (II) chloride dihydrate.

Stannous chloride-sulfuric acid TS See tin (II) chloride-sulfuric acid TS.

Stannous chloride TS See tin (II) chloride TS.

Stannous chloride TS, acidic See tin (II) chloride TS, acidic.

Starch [Special class].

Starch-sodium chloride TS Saturate starch TS with sodium chloride. Use within 5 to 6 days.

Starch, soluble [Special class].

Starch TS Triturate 1 g of starch with 10mL of cold water, and pour the mixture slowly, with constant stirring, into 200mL of boiling water. Boil the mixture until a thin, translucent fluid is obtained. Allow to settle, and use the supernatant liquid. Prepare before use.

Stearyl alcohol [Same as the namesake monograph in Part II].

Strong ammonia water See ammonia solution (28).

Strong cupric acetate TS See copper (II) acetate TS, strong.

Strong hydrogen peroxide water See hydrogen peroxide (30).

Strontium chloride $SrCl_2.6H_2O$ [Special class].

Styrene C_3H_8 Colorless, clear liquid.
Specific gravity between 0.902 and 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 by the area percentage method: not less than 99 %.

Operating conditions
Detector: A thermal conductivity detector.
Column: A glass column, about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth (180 to 250 m in particle diameter) coated with polyethylene glycol 20 mol/L at the ratio of 10 %.
Column temperature: A constant temperature of about 100 °C.
Temperature of sample vaporization chamber: A constant temperature of about 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 twice as long as the retention time of styrene.

Substrate solution for peroxidase determination Dissolve 0.195mL of hydrogen peroxidase(30), 8.38 g of disodium hydrogen phosphate 12-hydrate and 1.41 g of citric acid monohydrate in water to make 300mL. To 15mL of this solution add 13 mg of o-phenylenediamine dihydrochloride before use.

Succinic acid, anhydrous $C_4H_4O_3$ White or pale yellowish white crystals or flakes. It is odorless. Soluble in water, freely soluble in hot water, and sparingly soluble in ethanol (95).
Purity (1) *Chloride* not more than 0.005 %.
(2) *Iron* not more than 0.001 %.
Residue on ignition : not more than 0.10 % (1 g).
Content not less than 98.0 %.
Assay Dissolve about 1 g of anhydrous succinic acid, accurately weighed, in 50mL of water by warming, cool, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

EachmL of 1 mol/L sodium hydroxide VS
= 50.04 mg of $C_4H_4O_3$.

Sucrose $C_{12}H_{22}O_{11}$ [Same as the namesake monograph in Part II 「Purified Sucrose」]

Sudan Ⅲ $C_{22}H_{16}N_4O$ Red-brown powder. It dissolves in acetic acid (100) and in chloroform, and insoluble in water, in ethanol (95), in acetone and in ether.
Melting point : 170-190 °C

Sudan Ⅲ TS Dissolve 0.01 g of Sudam Ⅲ in 5mL of ethanol (95), filter, and add 5mL of glycerin to the filtrate. Prepare before use.

Sulbactam sodium for sulbactam penicillamine $C_8H_{10}NNaO_5S$ White to yellowish white crystalline powder. Freely soluble in water, and slightly soluble in ethanol (95).
Identification Determine the infrared absorption spectrum of sulbactam sodium for sulbactam penicillamine according to the potassium bromide disk method under the Infrared Spectrophotometry: it exhibit absorptions at the wavenumber 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 not more than 1.0 % (0.5 g)
Content not less than 875 μg per mg, calculated on the anhydrous basis.
Assay Weigh accurately an amount of sulbactam sodium for sulbactam penicillamine and Sulbactam Reference Standard, equivalent to about 0.1g (potency), dissolve each in a suitable volume of the mobile phase, add exactly 10mL of the intermal standard solution and the mobile phase to make 100mL, and use these solutions as the sample solution and the standard solutioin, respectively. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_t and Q_s, of the peak area of sulbactam to that of the internal standard.

Amount [μg (potency)] of sulbactam ($C_8H_{10}NNaO_5S$)
= amount [mg (potency)] of Sulbactam RS
$$\times \frac{Q_T}{Q_S} \times 1000$$

Interanal Standard solution A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

Operating conditions
Detector: Ultraviolet absorption photometer (wavelength: 220nm)
Column: A stainless steel column 3.9mm in inside diameter and 30cm in length, packed with octadecylsilanized silical gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35 °C.

Mobile phase: To 750mL of 0.005mol/L tetrabutylammmonium hydroxide TS add 250mL 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 performace: When the procedure is run with 10 μL of the standard solution according to the above operation conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution according to the above operating conditions, the relative standard deviation of the peak area of sulbactam is not more than 2.0 %

Sulfamic acid (standard reagent) See amido sulfuric acid (standard reagent).

Sulfanilamide $H_2NC_6H_4SO_2NH_2$ [Special class]

Sulfanilamide for titration of diaxotization $H_2NC_6H_4SO_2NH_2$ [For titration of diazotization]

Sulfanilic acid $H_2NC_6H_4SO_3H$ [Special class]

Sulfathiazole $C_9H_9N_3O_3S_2$ White crystalline powder.
Melting point 200 ~ 204 °C

5-Sulfosalicylic acid dihydrate $C_7H_6O_6S{\cdot}2H_2O$ [Special class]

Sulfosalicylic acid TS Dissolve 5 g of 5-Sulfosalicylic acid dihydrate in water to make 100mL

Sulfur S[Special class]

Sulfur dioxide SO_2 Preprae by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

Sulfuric acid H_2SO_4 [Special class]

Sulfuric acid, dilute Cautiously add 5.7mL of sulfuric acid to 10mL of water, cool, and dilote with water to make 100mL (10 %).

Sulfuric acid-ethanol TS With stirring add slowly 3mL of sulfuric acid to 1000mL of ethanol (99.5), and cool.

Sulfuric acid for readily carbonizable substances To sulfuric aicd, the content of which has previously been determined by the following method, add water cautiously, and adjust the final 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 abojt 2 g of sulfuric acid in a glass-stopped flask rapidly, add 30mL of water, cool, and titrate the solution with 1mol/L sodium hydroxide VS (indicator:2 to 3 drops of bromothymol blue TS).

EachmL of 1mol/L sodium hydroxide VS
= 49.04mg of H_2SO_4

Sulfuric acid, fuming $H_2SO_4{\cdot}nSO_3$ [Specialclass]

Sulfuric acid-methanol TS Prepare carefully by adding 60mL of sulfuric acid to 40mL of methanol.

Sulfuric acid-methanol TS, 0.05mol/L Add gradually 3mL of sulfuric acid to 1000mL of methanol, while stirring, and allow to cool.

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.

Sulfuric acid-sodium hydroxide TS With stirring add slowly 120mL of sulfuric acid to 1000mL of water, and cool (solution A). Dissolve 88.0 g of sodium hydroxide in 1000mL of freshly boiled and cooled water (solution B). Mix equal volumes of solution A and B.

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 100mL of sulfuric acid TS with water to make 1000mL.

Sulfuric acid TS, 0.05 mol/L Dilute 100mL of 0.5 mol/L sulfuric acid TS with water to make 1000mL.

Sulfuric acid TS, 0.25 mol/L With stirring, add slowly 15mL of sulfuric acid to 1000mL of water, then cool.

Sulfuric acid TS, 0.5 mol/L With stirring, add slowly 30mL of sulfuric acid to 1000mL of water, then cool.

Sulpyrine $C_{13}H_{16}N_3NaO_4S{\cdot}H_2O$ [Same as the name sake monograph].

Syntheric zeolite for drying 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 nm, and the surface area is 500 to 700 m2perg.
Loss on ignition not more than 2.0 % [2 g, between 550 and 600 °C , 4 hours, allow to stand in a desiccator (P_2O_5)].

Talc [Same as the namesake monograph in Part II].

Tannic acid [Same as the namesake monograph].

Tannic acid TS Dissolve 1 g of tannic acid in 1mL of ethanol (95), and add water to make 10mL. Prepare before use.

Tartaric acid See L-tartaric acid.

L-Tartaric acid $C_4H_6O_6$ [L(+)-Tartaricacid, Special class].

Tartaric acid buffer solution, pH 3.0 Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartarate dihydrate in water to make 1000mL.

Tellurium dioxide O_2Te White crystal
Melting point about 733 °C.

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 not more than 0.3 % (1 g).
Content not less than 95.0 %.
Assay Weigh accurately about 2 g of terephthalic acid, dissolve in exactly 50mL of 1 mol/L sodium hydroxide VS, and titrate with 1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make away necessary conection

EachmL of 1 mol/L sodium hydroxide VS
= 83.07 mg of $C_8H_6O_4$

Terphenyl $C_{18}H_{14}$ White, crystalline powder.
Melting point between 208 and 213 °C.
Identification Determine the absorption spectrum of a solution of terphenyl in methanol (1 in 250,000) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 276 and 280 nm.

p-Terphenyl See terphenyl.

Tetrabromophenolphthalein ethyl ester potassium salt $C_{22}H_{13}O_4Br_4K$[Special class].

Tetrabromo phenol phthalein ethyl ester TS Dissolve 0.1 g of tetrabromophenol phthalein ethyl ester potassium salt in acetic acid (100) to make 100mL. Prepare before use.

Tetrabutylammonium hydrogensulfate $C_{16}H_{37}NO_4S$ White crystalline powder. Soluble in alcohol, formig a colorless solution with a slight turbidity.
Melting point 169 ~ 173 °C
Assay Weigh accurately 170 mg of Tetrabutylammonium hydrogensulfate, dissolve in 40mL of water, and titrate potentiometrically with 0.1 mol/L hydrochloric acid VS. Perform a blank determination and make any necessary correction.

1mL of 0.1 mol/L ydrochloric acid VS.
= 33.95 mg of $C_{16}H_{37}NO_4S$

0.005 mol/L Tetrabutylammonium hydroxide TS To 10mL of tetrabutylammonium hydroxide TS add 700mL of water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000mL

Tetrabutylammoniam hydroxide-methanol TS Methanol solution containing 25 g/dL of tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$: 259.48]. Colorless to pale yellow solution, having an ammonium-like odor.
Content between 22.5 and 27.5 g/dL.
Assay Pipet 15mL of tetra butyl ammonium hydroxide-methanol TS and titrate with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

EachmL of 1 mol/L hydrochloric acid VS
= 259.48 mg of $C_{16}H_{37}NO$

10 % Tetrabutylammonium hydroxide-methanol TS Methanol solution containing 10 g/dL of tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$:259.48].
Content between 9.0 and 11.0 g/dL.
Assay Pipet 2mL of 10 % tetrabutylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

EachmL of 0.1 mol/L hydrochloric acid VS
= 25.948 mg of $C_{16}H_{37}NO$

Tetrabutylammonium hydroxide TS Solution containing 0.13 g/mL of tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$: 259.48].
Content between 11.7 and 14.3 g/dL.
Assay Pipet a quantity, equivalent to about 0.3 g of tetrabutylammonium hydroxide [$(C_4H_9)_4NOH$], transfer to a glass-stoppered flask containing 15mL of water, accurately weighed, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

EachmL of 0.1 mol/L hydrochloric acid VS
= 25.948 mg of $C_{16}H_{37}NO$

Tetracycline $C_{22}H_{24}N_2O_8$ [Same as the monograph Tetracycline].
Yellow to dark yellow, crystals or crystalline powder. Sparingly soluble in ethanol, and very slightly soluble in water.
Content : it contains not less than 870 mg (potency) per mg.
Assay. Proceed as directed in the Assay under Tetracycline Hydrochloride. However, use the following formula.

Amount [mg (potency)] of tetracycline ($C_{22}H_{24}N_2O_8$)
$= M_S \times (A_T/A_S) \times 1000$

M_S: Amount [mg (potency)] of Tetracycline Hydrochloride RS

Tetraethylammonium hydroxide TS $(C_2H_5)_4NOH$ [Tetraethylammonium hydroxide solution (10 %), Special class].

Tetrahydrofuran $CH_2(CH_2)_2CH_2O$ [Special class].

Tetrahydroxyquninone $C_6H_4O_6$ Dark blue crystals. Its color changes to yellow on exposure to light. Soluble in ethanol (95), and sparingly soluble in water.

Tetrahydroxyquinone indicator Mix 1 g of tetrahydroxyquinone with 100 g of sucrose homogeneously.

Tetramethylammonium hydroxide $(CH_3)_4NOH$ Ordinarily, available as an approximately 10 % aqueous solution, which is clear and colorless, and has a strong ammonia-like odor. Tetramethylammonium hydroxide is a stronger base than ammonia, and rapidly absorbs carbon dioxide from the air. Use a 10 % aqueous solution.
Purity Ammonia and other amines. Weigh accurately a quantity of the solution, corresponding to about 0.3 g of tetramethylammonium hydroxide [$(CH_3)4NOH$], in a weighing bottle already containing 5mL of water. Add a slight excess of 1 mol/L hydrochloric acid TS (about 4mL), and evaporate on a water bath to dryness. The mass of the residue (tetramethylammonium chloride), dried at 105 °C for 2 hours and multiplied by 0.8317, represents the quantity of tetramethylammonium hydroxide [$(CH_3)_4NOH$], and corresponds to ± 0.2 % of that found in the Assay.
Residue on evaporation : not more than 0.02 % (5mL, 105 °C, 1 hour).
Content : not less than 98z of the labeled amount.
Assay. Accurately weigh a glass-stoppered flask containing about 15mL of water. Add a quantity of the solution, equivalent to about 0.2 g of tetramethylammonium hydroxide [$(CH_3)_4NOH$], weigh again, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: methyl red TS).

EachmL of 0.1 mol/L hydrochloric acid VS
$=$ 9.115 mg of $C_4H_{13}NO$

Tetramethylammonium hydroxide-methanol TS Methanol solution containing of 10 g/dL of tetramethylammonium hydroxide [$(CH_3)_4NOH$: 91.15].
Content : between 9.0 and 11.0 g/dL.
Assay Pipet 2mL of tetramethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20mL of water, and titrate with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

EachmL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg of $C_4H_{13}NO$

Tetramethylammonium hydroxide TS, pH 5.5 To 10mL of tetramethylammonium hydroxide add 990mL of water, and adjust the pH to 5.5 with diluted phosphoric aicd (1 in 10).

Tetra n-pentylammonium bromide see Tetra n-pentylammonium bromide

Tetra n-butylammonium bromide $[CH_3(CH_2)_3]_4NBr$ White crystals or crystalline powder, having a slight, characteristic odor.
Melting point between 101 and 105 °C
Purity Clarity and color of solution Dissolve 1.0 g of tetra n-butylammonium bromide in 20mL of water: the solution is clear and colorless.
Content not less than 98.0 % .
Assay Dissolve about 0.5 g of tetra n-butylammonium bromide, accurately weighed, in 50mL of water, add 5mL of dilute nitric acid, and titrate with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

EachmL of 0.1 mol/L silver nitrate VS
= 32.237 mg of $C_{16}H_{36}NBr$

Tetra n-butylammonium chloride $C_{16}H_{36}ClN$ Whitecrystals,anditisdeliquescent.
Water not more than 6.0 % (0.1 g).
Content not less than 95.0 percent, calculated on the anhydrous basis.
Assay Weigh accurately about 0.25 g of tetra n-butylammonium chloride, dissolve in 50mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

EachmL of 0.1 mol/L silver nitrate VS
= 27.792 mg of $C_{16}H_{36}ClN$

Tetra-n-heptylammonium bromide $[CH_3(CH_2)_6]_4NBr$ White crystals, or crystalline powder, having a slight, characteristic odor.
Melting point Between 87 and 89 °C.

Tetraphenylboron potassium TS Add 1mL of acetic acid (31) to a solution of potassium biphthalate (1 in 500), then to this solution, add 20mL of a solution of tetraphenylboron sodium (7 in 1000), shake weil, and allow to stand for 1 hour. Collect the produced precipitate on filter paper, and wash it with water. To 1 /3 quantity of the precipitate, add 100mL of water, warm, with shaking, at about 50 °C for 5 minutes, cool quickly, allow to stand for 2 hours with occasional shaking, and filter, discarding the first 30mL of the filtrate.

Theophylline $C_7H_8N_4O_2$ White powder. Slightly soluble in water.
Melting point between 269 and 274 °C
Purity Caffeine, theobromine or paraxanthine To 0.20 g of theophylline add 5mL of potassium hydroxide TS or 5mL of ammonia TS: each solution is clear.
Loss on drying not more than 0.5 % (1 g, 105 °C, 4 hours).
Content not less than 99.0 %.
Assay Weigh accurately about 0.25 g of theophylline, previously dried, dissolve it in 40mL of N,N-dimethylformamide, and titrate with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-N,N-dimethylformamide TS). Perform a blank determination, and make any necessary correction.).

Eachml of 0.1 mol/L sodium methoxide VS
= 18.017 mg of $C_7H_8N_4O_2$

Thiamine nitrate $C_{12}H_{17}N_5O_4S$ [Same as the name-sake monograph].

Thiodiglycol $S(CH_2CH_2OH)_2$ [b-Thiodiglycol for amino acid autoanalysis] Colorless or pale yellow, clear liquid.
Specific gravity d_{20}^{20} between 1.180 and 1.190.
Water: not more than 0.7 %.

Thioglycolate medium I for sterility test See the Sterility Test under the General Tests, Processes and Apparatus.

Thioglycolate medium II for sterility test See the Sterility Test under the General Tests, Processes and Apparatus.

Thioglycolic acid See mercaptoacetic acid.

Thionyl chloride $SOCl_2$ [Special class].

Thiosemicarbazide $H_2NCSNHNH_2$ [Special class].

Thiourea H_2NCSNH_2 [Special class].

Thiourea TS Dissolve 10g of thiourea in water to make 100mL.

L-Threonine $C_4H_9NO_3$

Threoprocaterol hydrochloride $C_{16}H_{22}N_2O_3 \cdot HCl$ To procaterol hydrochloride, add 10 volumes of 3 mol/L hydrochloric acid TS, heat, and redux for 3 hours. After cooling, neutralize (pH 8.5) with sodium hydroxide TS, and collect the crystals produced. Suspend the crystals in water, dissolve by acidifying the solution at pH 1 to 2 with addition of hydrochloric acid, neutralize (pH 8.5) by adding sodium hydroxide TS, and separate the crystals produced. Suspend the crystals in 2-propanol, and acidify the solution at pH 1 to 2 by adding hydrochloric acid. The crystals are dissolved and reproduced. Collect the crystals, dry at about 60 °C while passing air. White to pale yellowish white, odorless crystals or crystalline powder.
Melting point About 207 °C (with decomposition).
Purity Dissolve 0.1 g of threoprocaterol hydrochloride in 100mL of diluted methanol (1 in 2), and use this solution as the test solution. Perform the test with 2 μL of the test solution as directed under the Liquid Chromatography according to the operating conditions in the Purity (3) under Procaterol Hydrochloride. Measure each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the area percentage method: it is not less than 95.0 %. Adjust the detection sensitivity so that the peak height of threoprocaterol obtained from 2 μL of a solution, prepared by diluting 5.0mL of the test solution with diluted methanol (1 in 2) to make 100mL, is 5 % to 10 % of the full scale, and the time span of measurement is about twice as long as the retention time of threoprocaterol after the peak of solvent.

Thrombin [Same as the name sake monograph in Part II].

Thymol $CH_3C_6H_3(OH)CH(CH_3)_2$ [Same as the name sake monograph in Part II].

Thymol blue $C_{27}H_{30}O_5S$ [Special class].

Thymol blue-*N,N*-dimethylformamide TS Dissolve 0.1g of thymolblue in 100mL of *N,N*-dimethyl formamide.

Thymol blue-dioxane TS Dissolve 0.05 g of thymol blue in 100mL of 1,4-dioxane, and filter, if necessary. Prepare before use.

Thymol blue TS Dissolve 0.1 g of thymol blue in 100mL of ethanol (95), and filter, if necessary.

Thymol blue TS, dilute Dissolve 0.05 g of thymol blue in 100mL of ethanol (99.5), and filter, if necessary. Prepare before use.

Thymolphthalein $C_{28}H_{30}O_4$ [Special class].

Thymolphthalein TS Dissolve 0.1 g of thymolphthalein in 100mL of ethanol (95), and filter, if necessary.

Tin Sn [Specialclass].

Tin (II) chloride dihydrate $SnC1_2.2H_2O$[Special class].

Tin (II) chloride-sulfuric acid TS Dissolve 10 g of tin (II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100mL.

Tin (II) chloride TS Dissolve 1.5 g of Tin (II) chloride di-hydrate in 10mL of water containing a small amount of hydrochloric acid. Preserve in glass-stoppered bottles 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 500mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.
Titanium dioxide Seetitanium (IV) oxide.

Titanium dioxide TS Seetitanium (IV) oxideTS.

Titanium (IV) oxide TiO_2[Special class].

Titanium (V) oxide TS To 100mL of sulfuric acid, add 0.1 g of titanium (IV) oxide, and dissolve by gradually heating on a liame with occasional gentle shaking.

Titanium trichloride See titanium (III) chloride.

Titanium trichloride-sulfuric acid TS See titanium (III) chloride-sulfuric acid TS.

Titanium trichloride TS See titanium (III) chlorideTS.

Titanium yellow $C_{28}H_{19}N_5Na_2O_6S_4$ [Special class].

Tocopherol $C_{29}H_{50}O_2$ [Same as the name sake monograph].

Tocopherol acetate $C_{31}H_{52}O_3$ [Same as the name sake monograph].

Tocopherol calcium succinate $C_{66}H_{106}CaO_{10}$ [Sameasthenamesakemonograph].

Tocopherol succinate $C_{33}H_{54}O_5$ Wet 0.5 g of tocopherol succinate with 5mL of acetic acid (100), add 10mL of toluene, and warm at 70 °C for 30 minutes with occasional shaking. After cooling, add 30mL of water, shake thoroughly, and allow to stand. Remove the water layer, wash the toluene layer with several 30mL volumes of water until the washings become neutral, and allow to stand. Shake the toluene extract with 3 g of anhydrous sodium sulfate, decant the toluene layer, distil the toluene under reduced pressure, and obtain a light yellow, viscous liquid. When preserved at room temperature for a long time, it becomes a pale yellowish solid.
Absorbance $E_{1\,cm}^{1\%}$ (286 nm) between 38.0 and 42.0 (0.01 g, chloroform, 100mL).

Tolbutamide $C_{12}H_{18}NO_3S$ [Same as the namesake monograph]

***p*-Tolualdehyde** C_8H_8O Colorless to yellow, clear liquid.
Content not less than 98 %.
Assay Perform the test with 5 % Carbon disulfide solution as directed under the Gas Chromatography according to the following conditions

Operating conditions
Detector: A flame-ionization detector
Column: A stainless steel column 3 mm in inside diameter and 1.8 m in length, packed with a 5 % phase diethylene gycol succinate polyesterl for gas chromatography on Siliceous earth for gas chromatography
Column temperature: about 125 °C,
Temperature of sample vaporization chamber: about 205 °C
Carrier gas: Nitrogen
Flow rate: Adjust the flow rate of about 12mL per minute
Refractive index n_D^{20} between 1.544 and 1.546.

Toluene $C_{12}H_{18}N_2O_3S$ [Special class].

o-Toluene sulfonamide $C_7H_9NO_2S$ Colorless crystals or white, crystalline powder. Soluble in ethanol (95), and sparingly soluble in water.
Melting point between 157 and 160 °C
Purity p-Toluene sulfonamide Use a solution of o-toluene sulfonamide in ethyl acetate (1 in 5000) as the test solution. Perform the test with 10 μL of the test solution as directed under the Gas Chromatography according to the operating conditions as directe in the Purity (6) under Saccharin Sodium: any peak other than the peak of o-toluene sulfonamide does not appear. Adjust the flow rate so that the retention time of o-toluene sulfonamide is about 10 minutes, and adjust the detection sensitivity so that the peak height of o-toluene sulfonamide obtained from 10 μL of the test solution is about 50 % of the full scale. Time span of measurement is about twice as long as the retention time of o-toluene sulfonamide after the solvent peak.
Water not more than 0.5 % (4 g, use 25mL of methanol for Karl Fischer method and 5mL of pyridine for Karl Fischer method).
Content not less than 98.5 percent, calculated on the anhydrous basis.
Assay Weigh accurately about 0.025 g of o-tol-uene sulfonamide, and perform the test as directed under the Nitrogen Determination.

EachmL of 0.005 mol/L sulfuric acid VS
= 1.7122 mg of $C_7H_9NO_2S$

***p*-Toluene sulfonamide** $CH_3C_6H_4SO_2NH_2$ White crystals or crystalline powder.
Melting point about 137 °C
Purity Related substances Dissolve 0.030 g of p-toluene sulfonamide in acetone to make exactly 200mL. Proceed with 10 μL of this solution as directed in the Purity (3) under Tolazamide: any spot other than the principal spot at the Rf value of about 0.6 does not appear.

***p*-Toluene sulfonic acid** See p-toluenesulfonicacid monohydrate.

***p*-Tolueaesulfonic acid monohydrate** $CH_3C_6H_4SO_3H \cdot H_2O$ [Special class].

***p*-Toluic Acid** $CH_3C_6H_4COOH$ White, crystalline powder. Sparingly soluble in hot water; very soluble in alcohol, in methanol, and in ether.
Assay Transfer about 650 mg, accurately weighed, to a suitable container, dissolve in 125mL of alcohol, add 25mL of water, and mix. Titrate with 0.5 mol/L sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction.

EachmL of 0.5 N sodium hydroxide
= 68.07 mg of $C_8H_8O_2$:

Content not less than 98 % .
Melting point 181 ~ 183 °C

Toluidine blue $C_{15}H_{16}ClN_3S$ Dark green powder, soluble in water, and slightly soluble in ethanol (95).

Tragacantha [Same as the name sake monograph in part II].

Triamcinolone acetonide $C_{24}H_{31}FO_6$ [Same as the namesake monograph].

Tributyrin (GlycerylTributyrate) $C_{15}H_{26}O_6$ Colorless, oily liquid. Insoluble in water; very soluble in alcohol and in ether.
Assay Perform the test with Trinutyrin as directed under the Gas Chromatography according to the following conditions.

Operating conditions
Detector: A flame-ionization detector
Column: A stainless steel column 3 mm in inside diameter and 1.8 m in length, packed with a 5 % phase diethylene gycol succinate polyesterl for gas chromatography on Siliceous earth for gas chromatography
Injection port temperature: about 270 °C,
Detector temperature: about 300 °C
Carrier gas: Nitrogen

Trichloroacetic acid $CC1_3COOH$ [Special class].

1,1,2-Trichloro-1,2,2-trifluoroethane $CFCl_2 \cdot CF_2Cl$ Colorless, volatile liquid. Miscible with acetone or diethyl ether, and not with water.
Purity Related substances Perform the test with 0.1 μL of 1,1,2-trichloro-1,2,2-trifluroethane as directed under the Gas Chromatography according to the operating conditions for Purity (5) under Halothane: any peak other than the peak of 1,1,2-trichloro-1,2,2-trifluoroethane does not appear.

Triethanolamine See 2,2',2"-nitrilotrisethanol.

Triethyl amine $(C_2H_5)_3N$ Clear, colorless liquid, having a strong amines odor. Miscible with methanol, with ethanol (95) or diethyl ether.

Triethylamine-phosphate buffer solutin, pH 5.0 To 1mL of triethylamine add 900mL of water, adjust the pH to 5.0 with diluted phosphoric acid (1 in 10), and add water to make 1000mL.

Trifluroacetic aicd CF_3COOH Colorless, clear liquid, having a pungent odor. Miscible well with water.
Boiling point between 72 and 73 °C
Specific gravity d_{20}^{20} 1.535

Trifluroacetic aicd TS To 1mL of trifluroacetic aicd add water to make 1000mL.

Trimethylsilyl imidazole $C_6H_{12}N_2Si$ Clear, colorless to pale yellow liquid.
Refractive index n_D^{20} between 1.4744 and 1.4764.

2,4,6-Trinitrobenzenesulfonic acid $C_6H_2(NO_2)_3SO_3H \cdot 2H_2O$ Pale yellow to light yellow powder.
Water 11-15 % (0.1g, volumetric titration, direct titration).
Content not less than 98 %, calculated on the anhydrous basis.
Assay Weigh accurately about 0.3 g of 2,4,6-trinitrobenzenesulfonic acid, dissolve in 50mL of a mixture of water and ethanol(99.5) (1 : 1), and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titiration). Perform a blank determination and make any necessary correction

EachmL of 0.1mol/L sodium hydroxide VS
= 29.317 mg of $C_6H_2(NO_2)_3SO_3H \cdot 2H_2O$

2,4,6-Trinitrophenol $HOC_6H_2(NO_2)_3$ [Special class]. Preserve in tight containers, in a cold place, remote from fire.

2,4,6-Trinitrophenol-ethanol TS Dissolve 1.8 g of 2,4,6-trinitrophenol in 50mL of diluted ethanol (99.5) (9 in 10) and 30mL of water, and add water to make 100mL.

2,4,6-Trinitrophenol TS Dissolve 1 g of 2,4,6-trinitro-phenol in 100mL of hot water, cool, and filter, if necessary.

2,4,6-Trinitropbenol TS, alkaline Mix 20mL of 2,4,6-trinitrophenol TS with 10mL of a solution of sodium hydroxide (1 in 20), and add water to make 100mL. Use within 2 days.

Triphenylchloromethane $(C_6H_5)_3CCl$ [Special class].

2,3,5-Tripheayl-2H-tetrazolium chloride $C_{19}H_{15}ClN_4$ [Special class].

2,3,5-Triphenyl-2H-tetrazolium chloride TS Dissolve 0.25 g of 2,3,5-triphen Dissolve 0.25 g of 2,3,5-triphenyl-2H-tetrazolium chloride in ethanol (99.5) to make 100mL. Prepare before use.

Tris buffer solution, pH 7.0 Dissolve 24.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000mL of water, and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid TS.

0.05 mol/L Tris buffer solution, pH 7.0 Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in about 750mL of water, and adjust the pH to 7.0 with 1 mol/L hydrochloric acid TS., and add water to make 1000mL.

0.1 mol/L Tris buffer solution, pH 8.0 Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 100mL of water, and adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200mL.

Tris buffer solution, pH 9.5 Dissolve 36.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000mL of water, and adjust the pH to 9.5 by adding 1 mol/L hydrochloric acid TS.

Trishydroxymethylaminomethane See 2-amino-2-hydroxymethy1-1,3-propanediol.

Trisodium citrate dehydrate See sodium citrate hydrate.

Trypsin inhibitor Produced by purifying soybeen. Each mg of trypsin inhibitor inhibits 10000 to 30000 BAEE Units of trypsin. One BAEE Unit means a trypsin activity to indicate an absorbance difference of 0.001 at 253 nm when 3.2mL of the solution is reacted at 25 °C and pH 7.6, using N- α -benxoyl-L-arginine ethyl ester as substarate

Trypsin inhibitor TS Dissolve 5mg of trypsin inhibitor in 0.05 mol/L phosphate buffer solution, pH 7.0 to make 10mL.

Trypsin TS for test of elcatonin Dissolve 5 mg of trypsin for liquid chromatography in 20mL of a solution of ammonium hydrogen carbonate (1 in 100). Prepare before use.

L-Tryptophan $C_{11}H_{12}N_2O_2$ [Same as the name sake monograph].

Turmeric paper Macerate 20 g of powdered turmeric, the dried root of *Curcuma longa* Linné, with four 100mL-volumns of cold water, decant the supernatant liquid each time, and discard it. Dry the residue at a temperature not over 100 °C. Macerate the dried residue with 100mL of ethanol (95) for several days, and filter. Immerse filter paper in this ethanol decoction, and allow the ethanol (95) to evaporate spontaneously in clean air.
Sensitivity Dip a strip of turmeric paper, about 1.5 cm length, in a solution of 1 mg of boric acid in a mixture of 1mL of hydrochloric acid and 4mL of water, after 1 minute, remove the paper from the liquid, and allow it to dry spontaneously: the yellow color changes to brown. When the strip is moistened with ammonia TS, the color of the strip changes to greenish black.

Turpentine oil [Same as the name sake monograph in Part II].

L-Tyrosin $C_9H_{11}NO_3$
White, crystals or crystalline powder. Odorless and tasteless. Freely soluble in formic acid, very slightly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute nitric acid.
Optical rotation $[\alpha]_D^{20}$: −10.5 !−12.5(after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50mL, 100 mm).
Loss on drying : not more than 0.30 % (1 g, 105 °C, 3 hours).
Content: not less than 99.0 %.
Assay Weigh accurately about 0.3 g of L-tyrosine, previously dried, dissolve in 6mL of formic acid, add 50mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

EachmL of 0.1 mol/L perchloric acid VS
= 18.12 mg of $C_9H_{11}NO_3$

Ubiquinone-9 A yellow to orange, crystalline powder. Odor less and no taste.
Melting point about 44 °C
Absorbance $E_{1\,cm}^{1\%}$ (275 nm) between 163 and 190 (ethanol (99.5)).

Uranyl acetate See uranyl acetate dihydrate.

Uranyl acetate TS Dissolve 1 g of uranyl acetate dihydrate in water to make 20mL, and filter, if necessary.

Uranyl acetate-zinc TS Dissolve 10 g of uranyl acetate dihydrate in 5mL of acetic acid (31) and 50mL of water by heating. Separately, dissolve 30 g of zinc acetate dihydrate in 3mL of acetic acid (31) and 30mL of water by heating. While the two solutions are still warm, mix them, cool, and filter.

Urea H_2NCONH_2 [Special class].

Urethane See ethyl carbamate.

n-Valerianic acid $CH_3(CH_2)_3COOH$ Clear and colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and with diethyl ether, and soluble in water.
Specific gravity between 0.936 and 0.942.
Distilling range between 186 and 188 °C, not less than 98 vol %.

L-Valine $C_5H_{11}NO_2$ [Same as namesake monograph]

Vanadium pentoxide See vanadium (V) oxide.

Vanadium pentoxide TS, dilute See vanadium (V) oxide TS, dilute.

Vanadium (V) oxide V_2O_5 [Special class]. Orangish yellow to yellow brown powder.
Identification. Dissolve 0.3 g in 10mL of ammonia TS and 15mL of water. To 2mL of this solution add 20mL of water, mix, and add gently 1mL of copper (II) sulfate TS: yellow precipitates appear.

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 Dilute 10mL of vanadium (V) oxide TS with water to make 100mL. Prepare before use.

Vanillin $C_6H_3CHO(OCH_3)(OH)$. A white to light yellow crystalline powder, having a characteristic odor.
Melting point : 80.5 ~ 83.5 °C.
Preserve in a light-resistant tight container.

Vanillin-hydrochloric acid TS Dissolve 5 mg of vanillin in 0.5mL of ethanol (95), and to this solution, add 0.5mL of water and 3mL of hydrochloric acid. Prepare before use.

Vanillin-sulfuric acid-ethanol TS Dissolve 3 g of vanillin in dehydrated ethanol (95) to make 100mL, and add 0.5mL of sulfuric acid.

Vanillin-sulfuric acid TS Add cautiously 75mL of sulfuric acid to 25mL of ice-cold ethanol (95). After cooling, add 1 g of vanillin to dissolve.

Vasopressin $C_{46}H_{65}N_{15}O_{12}S_2$ A white powder.
Constituent amino acids. Perform the test as directed in the Constituent amino acids under Oxytocin, and calculate the respective molar ratios 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 not more than 0.03 for other amino acids.

Vegetable oil Vegetative oils specified in monographs

Vinblastine sulfate $C_{46}H_{58}N_4O_9.H_2SO_4$ [Same as the namesake monograph].

Vincristine sulfate $C_{46}H_{56}N_4O_{10}.H_2SO_4$ [Same as the namesake monograph].

Vinyl acetate $C_4H_6O_2$ Clear, colorless liquid.
Specific gravity d_{20}^{20} between 0.932 and 0.936.
Water not more than 0.2 %.

1-Vinyl-2-pyrrolidone C_6H_9NO Clearliquid.
Purity Perform the test with 0.5 μL of 1-vinyl-2-pyrrolidone as directed under the Gas Chromatography according to the following conditions. Determine each peak area of the solutions by the automatic integration method, and calculate the amount of 1-vinyl-2-pyrrolidone by the area percentage method: it is not less than 99.0 %.

Operating conditions
Detector: A hydrogen flame-ionization detector.
Column: A hollow, capillary glass column about 0.53 mm in inside diameter and about 30 m in length, having an about 1.0 μm layer of polyethylene glycol 20 M for gas chromatography on the inner side.
Column temperature: Maintain the temperature at 80 °C for 1 minute, then raise at the rate of 10 °C per minute to 190 °C , andholdconstanttothetemperature for 20minutes.
Temperature of sample vaporization chamber: A constant temperature of about 190 °C.
Carrier gas: Helium
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 from 0.5 μL of l-vinyl-2-pyrrolidone composes about 70 % of the full scale.
Time span of measurement: About twice as long as the retention time of 1-vinyl-2-pyrrolidone.
Water Take 50mL of methanol for Karl Fischer Method and 10mL of butyrolactone in a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 2.5 g of 1-vinyl-2-pyrrolidone, transfer immediately to a titration flask, and perform the test: water is not more than 0.1 %.

25 % Water containing benzoyl peroxide See Benzoylper oxide, 25 % water containing.

Water for bacterial endotoxins test [Same as the monograph Water for Injection or water produced by

other procedures that shows no reaction with the LAL reagent employed, at the detection limit of the reagent.].

Water for injection [Same as the namesake monograph in Part II].

Wijs' TS Transfer 7.9 g of iodine trichloride and 8.9 g of iodine to separate flasks, dissolve each with acetic acid (100), mix both solutions, and add acetic acid (100) to make 1000mL. Preserve in light-resistant, glass containers.

Xanthone $C_{13}H_8O_2$ Light yellow powder. Freely soluble in chloroform, and slightly soluble in hot water or diethyl ether.
Melting point between 174 and 176 °C.
Purity Related substances Dissolve 0.050 g of xanthone in chloroform to make exactly 10mL. Perform the test with 5 μL of this solution as directed in the Purity under Propantheline Bromide: any spot other than the principal spot at the Rf value of about 0.7 does not appear.

Xylene $C_6H_4(CH_3)_2$ [First class].

o-Xylene $C_6H_4(CH_3)_2$ Colorless, clear liquid.
Refractive index n_D^{20} between 1.501 and 1.506.
Specific gravity d_{20}^{20} between 0.875 and 0.885.
Distilling range 143 – 146 °C, not less than 95 vol %.

Xylene cyanol FF $C_{25}H_{27}N_2NaO_7S_2$ [Special class].

Xylenol orange $C_{31}H_{30}N_2Na_2O_{13}S$ [Special class].

Xylenol orange TS Dissolve 0.1g of xylenol orange in water to make 100mL.

Xylitol $C_5H_{12}O_5$ [Same as the name sake monograph].

Xylose See D-xylose.

D-Xylose $C_5H_{10}O_5$ [Same as the monograph D-Xylose of the Korean Standards of Food Additives].

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. Yeast extract (1 g) represents not less than 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)* : not more than 5 %. (2) *Coagulable protein* On heating a solution of yeast extract (1 in 20) to boiling, no precipitate is produced.
Loss on drying not more than 5 % (105 °C, constant mass).
Residue on ignition not more than 15 % (0.5 g).
Nitrogen content between 7.2 and 9.5 % (105 °C constant mass, after drying, according to the Nitrogen Determination).

Yellow beeswax [Same as the namesake monograph in Part II].

Yellow mercuric oxide See mercury (II) oxide, yellow.

Yellow mercury (II) oxide See mercury (II) oxide, yellow.

Zinc Zn [Special class].

Zinc (Standard reagent) Zn [Special class].

Zinc for arsenic analysis Zn [K8012] Use granules of about 800mm.

Zinc acetate See zinc acetate dihydrate.

Zinc acetate dihydrate $Zn(CH_3COO)_2.2H_2O$ [Special class].

Zinc, arsenic-free See zinc for arsenic analysis.

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 1000mL.

Zinc iodide-starch paper Impregnate the filter paper for volumetric analysis with freshly prepared zinc iodide-starch TS, and dry in the clean room. Preserve in a glass-stoppered bottle, protected from light and moisture.

Zinc iodide-starch TS To 100mL of boiling water add a solution of 0.75 g of potassium iodide in 5mL of water, a solution of 2 g of zinc chloride in 10mL of water and a smooth suspension of 5 g of starch in 30mL of water, with stirring. Continue to boil for 2 minutes, then cool.
Sensitivity Dip a glass rod into a mixture of 1mL of 0.1 mol/L sodium nitrite VS, 500mL of water and 10mL of hydrochloric acid, and touch on zinc iodide-starch paste TS: an apparently blue color appears.
Storage Preserve in tightly stoppered bottles, in a cold place.

Zincon $C_{20}H_{16}N_4O_6S$ 1-(2-hydroxy-5-sulfophenyl)-3-phenyl-5-(2-carboxyphenyl)formazan A red-purple powder. Soluble in sodium hydroxide TS, forming a red solution.
Melting point 210 ~ 220 °C

Zincon TS Dissolve 0.1 g of zincon in 2mL of 1 mol/L sodium hydroxide VS, and add water to make 100mL.

Zinc powder Zn [Special class]

Zinc sulfate See zinc sulfate heptahydrate.

Zinc sulfate for volumetric analysis See zinc sulfate heptahydrate.

Zinc sulfate heptahydrate $ZnSO_4 \cdot 7H_2O$ [Special class]

Zinc sulfate TS Dissolve 10 g of zinc sulfate heptahydrate in water to make 100mL.

Zirconyl-alizarin S TS Seezirconyl-alizarinred S TS. Dissolve 0.2 g of zirconyl nitrate in 5mL of dilute hydrochloric acid, add 10mL of alizarin red S TS, and then add water to make 30mL.

Zirconyl nitrate See zirconyl nitrate dihydrate.

Zirconyl nitrate dihydrate $ZrO(NO_3)_2 \cdot 2H_2O$ [Special class]

*** Solid Supports/Column Packings for Chromatography**

Alkylene glycol phthalate ester for gas chromatography Prepared for gas chromatography.

Aminopropylsilicated silica gel for liquid chromatography Prepared for liquid chromatography

7 % Cyanopropyl-7 % phenylmethylsilicon polymer for gas chromatography Prepared for gas chromatography

Cyanopropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Diethylene glycol adipinate for gas chromatography Prepared for gas chromatography.

Diethylene glycol succinate ester for gas chromatography Prepared for gas chromatography.

Dimethylaminopropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

1 % Dimethyl polysiloxan rubber for gas chromatography Prepared for gas chromatograph

Fluorosilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linkage: 8 %) Prepared for liquid chromatography.

Gel type strong acid ion-exchange resin for liquid chromatography (degree of cross-linkage: 6 %) Prepared for liquid chromatography.

Hydroxy propylsilanized silca gel for liquid chromatography Prepared for liquid chromatography.

Methylsilicone polymer for gas chromatography Prepared for gas chromatography.

Neutral alumina for column chromatography Prepared for column chromatography.

Octadecylsilanized polyvinyl alcohol gel polymer for liquidchromatography Prepared for liquid chromatography.

Octadecylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized silicone polymer coated silica gel for liquid chromatography Prepared for liquid chromatography.

Octylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Penta ethylene hexaaminate polyvinyl alcohol polymer bead for liquid chromatography Prepared ior liquid chromatography.

Phenylated silica gel for liquid chromatography Prepared for liquid chromatography.

35 % Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography

50 % Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography

65 % Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography

25 % Phenyl-25 % cyanopropyl-methylsilicone polymer for gas chromatography Prepared for gas chromatography

Polyalkylene glycol monoether 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 400 for gas chromatography Prepared for gas chromatography

Polyethylene glycol 6000 for gas chromatography Prepared for gas chromatography

Porous acrylnitril-divinylbenzene copolymer for gas chromatography (porediameter: 0.06-0.08μm, 100-200m^2/g) A porous acrylnitril-divinylbenzene copolymer prepared for gas chromatography.

Porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0075 μm, 500 -600m^2/g) A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075 μm, and surface area is 500 - 600 m2 per g.

Porous polymer beads for gas chromatography Prepared for gas chromatography

Porous silica gel for liquid chromatography A porous silica gel prepared for liquid chromatography.

Silica gel for gas chromatography A silica gel prepared for gas chromatography.

Silica gel for liquid chromatography A silica gel prepared for liquid chromatography.

Silica gel for thin-layer chromatography A silica gel prepared for thin-layer chromatography.

Silica gel with complex fluorescent indicator for thin-layerchromatography A silica gel for thin-layer chromatography containing suitable complex fluorescent indicators.

Silica gel with fluorescent indicator for thin-layerchromatography A silica gel for thin-layer chromatography containing a suitable fluorescent indicator.

Siliceous earth [First class].

Siliceous earth for chromatography A siliceous earth prepared for chromatography.

Siliceous earth for gas chromatography A siliceous earth prepared for gas chromatography.

D-Sorbitol for gas chromatography Prepared for gas chromatography.

Strongly acidic ion exchange resin

Strongly acidic ion exchange resin for column chromatography Prepared for column chromatography.

Strongly acidic ion exchange resin for liquid chromatography Prepared for liquid chromatography.

Terephthalic acid for gas chromatography $C_6H_4{\cdot}(COOH)_2$ Terephthalic acid prepared for gas chromatography.

Trimetylsilanized silica gel for liquid chromnatography Prepared for liquid chromatography.

Zeolite for gas chromatograpby (pore diameter 0.5 nm) Zeolite prepared for gas chromatography.

(3) Standard Solutions for Volumetric Analysis

Standard solutions for volumetric analysis are prepared to a specified molar concentration. An 1 molar solution is a solution which contains exactly 1 mole of the reagent in each 1000mL of the solution and is designated to be 1 mol/L. When necessary, these solutions may be diluted to specified molar concentrations and the diluted solutions can be used as standard solutions. For example, 0.1 mol/L solution is obtained by diluting 1 mol/L solution 10 times by volume.

Unless otherwise specified, standard solutions for volumetric analysis should be stored in colorless or light-resistant, glass-stoppered bottles.

Preparation and Standardization

A volumetric standard solution may be prepared by one of the following methods. The degree of difference from a specified concentration, *n* (mol/L),is expressed as a factor (molar concentration coeflicient) *f*. Usually, standard solutions are prepared to obtain *f* in the range of 0.970-1.030. The determination procedure for *f* is called standardization of the standard solution.

(1) Weigh accurately a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the pure substance, and dissolve it in the specified solvent to make exactly 1000mL to prepare a standard solution having a concentration close to the specified molarity, *n* (mol/L). In this preparation, the factor *f* of the standard solution is obtained by dividing the g mass of the pure substance by the molecular mass of the substance (m) and the specified molarityn umber, *n*. When a pure substance is not obtain able, a highly purified substance, whose purity has been exactly determined and certified, may be used.

(2) In the case where a pure substance or a highly purified substance is not obtainable, weigh a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the substance specified for each standard solution and dissolve it in the specified solvent to make about 1000mL to prepare a standard solution having a concentration close to the specified molarity, *n* (mol/L). The factor, f of this solution is deter-

mined by applying the standardization procedure described for the standard solution. Directand in direct methods may be used for the standardization:

a) Direct method: Weigh accurately a standard reagent or an indicated substance specified for each standard solution, dissolve it in the specified solvent, then titrate with the prepared standard solution to be standardized, and determine the factor, f,byapplyingthefollowingequation.

$$f = \frac{1000 \cdot m}{V \cdot M \cdot n}$$

M: Molecular mass equivalent to 1 mole of the standard reagent or the specified substance (g)

m: Mass of the standard reagent or the specified substance taken (g),

V: Volume of the prepared standard solution consumed for the titration (mL), and

n: Arithmetic number of mole for the specified molar concentration of the standard solution for standardized (e.g. n = 0.02 for 0.02 mol/L standard solution).

b) Indirect method When an appropriate standard reagent is not available, titrate a defined volume V_2 (mL) of a standard solution to be standardized with the specified standard solution having a known factor (f_1), and calculate the factor (f_2) by applying the following equation

$$f_2 = \frac{V_1 \cdot f_1}{V_2}$$

f_1:Factor for the titrating standard solution with a known factor,

f_2: Factor for the prepared standard solution to be standardized,

V_1: Volume of the titrating standard solution consumed (mL), and

V_2: Volume of the prepared standard solution taken (mL).

(3) Standard solutions are prepared by diluting exactly an accurately measured volume of a standard solution having a known factor, according to the specified dilution procedure. During this dilution procedure, the original factor of the standard solution is assumed constant.

Ammonium Iron (II) Sulfate, 0.1 mol/L

1000mL of this solution contains 39.214 g of ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$: 392.14].

Preparation In a cooled mixture of 30mL of sulfuric acid and 300mL of water, dissolve 40 g of ammonium iron (II) sulfate hexahydrate. Dilute the solution with water to make 1000mL, and standardize by the following method:

Standardization To exactly 25mL of the prepared ammonium iron (II) sulfate solution, add 25mL of water and 5mL of phosphoric acid. Titrate the solution with 0.02 mol/L potassium permanganate VS. Calculate the molarity factor.

Note Prepare before use.

Ammonium Iron (II) Sulfate, 0.02 mol/L

1000mL of this solution contains 7.843 g of ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$: 392.14].

Preparation Prior to use, dilute 0.1 mol/L ammonium Iron (II) sulfate VS with diluted sulfuric acid (3 in 100) to make exactly 5 times the initial volume.

Ammonium Iron (III) Sulfate, 0.1 mol/L

1000mL of this solution contains 48.22 g of ammonium iron (III) sulfate 12-hydrate [$FeNH_4(SO_4)_2 \cdot 12H_2O$: 482.19].

Preparation Dissolve 49 g of ammonium iron (III) sulfate 12-hydrate in a cooled mixture of 6mL of sulfuric acid and 300mL of water, add water to make 1000mL, and standardize the solution as follows:

Standardization Measure exactly 25mL of the prepared ammonium iron (III) sulfate solution into an iodine flask, add 5mL of hydrochloric acid, and shake the mixture. Dissolve 2 g of potassium iodide, and stopper the flask. After standing the mixture for 10 minutes, add 50mL of water, and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS. When a pale yellow color, indicating the imminent end point, appears, add 3mL of starch TS. Continue the titration, until the blue color disappears. After a blank determination, calculate the molarity factor.

Note Store protected from light. After a prolonged storage, this solution requires restandardization.

Ammonium Thiocyanate, 0.1 mol/L

1000mL of this solution contains 7.612 g of ammonium thiocyanate (NH_4SCN: 76.12).

Preparation Dissolve 8 g of ammonium thiocyanate in water to make 1000mL, and standardize the solution as follows:

Standardization Measure exactly 25mL of the 0.1 mol/L silver nitrate VS, and add 50mL of water, 2mL of nitric acid, and 2mL of ammonium iron (III) sulfate TS. Titrate the solution with the prepared ammonium thiocyanate solution to the first appearance of a persistent red-brown color with shaking. Calculate the molarity factor.

Note Store protected from light.

Ammonium Thiocyanate, 0.02 mol/L

1000mL of this solution contains 1.5224 g of ammonium thiocyanate (NH_4SCN: 76.12)

Preparation Before use, dilute 0.1mol/L ammonium thiocyanate VS with water to make exactly 5 times the initial volume.

Barium chloride, 0.1 mol/L

1000mL of this solution contains 24.426 g of barium chloride dihydrate ($BaCl_2.2H_2O$: 244.26).

Preparation. Dissolve 24.5 g of barium chloride dihydrate in water to make 1000mL, and standardize the solution as follows:
Standardization. Measure exactly 20mL of the prepared solution, add 3mL of hydrochloric acid, and warm the mixture. Add 40mL 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 the mixture, wash the precipitate on the filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat again at about 700 °C for 2 hours. After cooling, weigh accurately the mass of the residue, and calculate the molarity factor as barium sulfate ($BaSO_4$).

EachmL of 0.1 mol/L barium chloride VS
= 23.34 mg of $BaSO_4$

Barium Chloride, 0.02 mol/L

1000mL of this solution 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 1000mL, and standardize the solution by the following method:
Standardization To exactly 100mL of the prepared barium chloride solution, add 3mL of.hydrochloric acid, and warm the mixture. Add 40mL of pre-warmed, diluted sulfuric acid (1 in 130), heat the mixture on a water bath for 30 minutes, and allow to stand overnight. Filter the mixture, wash the collected filtrate with water until the washing shows no turbidity upon the addition of silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat strongly again at about 700 °C for 2 hours. After cooling, weigh accurately the residue as barium sulfate ($BaSO_4$), and calculate the molarity factor.

EachmL of 0.02 mol/L barium chloride VS
= 4.668 mg of $BaSO_4$

Barium Chloride, 0.01 mol/L

1000mL of this solution contains 2.4426 g of barium chloride dihydrate $BaCl_2 \cdot 2H_2O$:244.26).
Preparation Prior to use, dilute 0.02 mol/L barium chloride VS with water to make exactly twice the initial volume.

Barium Perchlorate, 5 mmol/L

1000mL of this solution contains 1.6812 g of barium perchlorate [$Ba(ClO_4)_2$: 336.23].
Preparation Dissolve 1.7 g of barium perchlorate in 200mL of water, dilute with 2-propanol to make 1000mL, and standardize the solution by the following method:
Standardization To exactly 20mL of the prepared barium perchlorate solution, add 55mL of methanol and 0.15mL of arsenazo III TS. Titrate the solution with 0.005 mol/L sulfuric acid VS until the purple color changes through red-purple to red. Calculate the molarity factor.

Bismuth Nitrate, 0.01 mol/L

1000mL of this solution contains 4.851 g of bismuth nitrate pentahydrate [$Bi(NO_3)_3 \cdot 5H_2O$: 485.07).
Preparation To 60mL of diluted nitric acid, dissolve 4.86 g of bismuth nitrate pentahydrate. Add water to the solution to make 1000mL, and standardize the solution by the following method:
Standardization Measure exactly 25mL of the prepared bismuth nitrate solution, add 50mL of water and 1 drop of xylenol orange TS, and titrate the solution with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the red color changes to yellow. Calculate the molarity factor.

Bromine, 0.05 mol/L

1000mL of this solution 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 1000mL, and standardize the solution as follows:
Standardization Measure exactly 25mL of the prepared solution into an iodine flask. Add 120mL of water, quickly add 5mL of hydrochloric acid, stopper the flask immediately, and shake it gently. Then add 5mL of potassium iodide TS, restopper immediately, shake the mixture gently, and allow to stand for 5 minutes. Titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS. When a pale yellow collor, indicating the imminent end point, appears, add 3mL of starch TS. Continue the titration, until the blue color disappears. After a blank determination, calculate the molarity factor.

Ceric Ammonium Sulfate, 0.1 mol/L

See cerium (IV) tetraammonium sulfate, 0.1 mol/L.

Ceric Ammonium Sulfate, 0.01 mol/L

See cerium (IV) tetraammonium sulfate, 0.01 mol/L.

Cerium Sulfate, 0.1 mol/L

1000mL of this solution contains 33.22 g of cerium sulfate [$Ce(SO_4)_2$: 332.24]
Preparation Use commercially available volumetric standard solution. Standardize the solution as follows.
Standardization Accurately weigh about 0.2 g of sodium oxalate, primary standard, dried according to the instructions on its label, and dissolve in 75mL of water. Add, with stirring, 2mL of sulfuric acid that has previously been mixed with 5mL of water, mix well, add 10mL of hydrochloric acid, and heat to between

70 °C and 75 °C. Titrate with 0.1 N ceric sulfate to a permanent slight yellow color.

EachmL of 0.1 mol/L cerium sulfate VS
= 6.700 mg of $Na_2C_2O_4$

Cerium (IV) Tetraammonium Sulfate, 0.1 mol/L

1000mL of this solution contains 63.26 g of cerium (IV) tetraammonium sulfate dihydrate [$Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$: 632.55].

Preparation In 0.5 mol/L sulfuric acid VS, dissolve 64 g of cerium (VI) tetraammonium sulfate dihydrate to make 1000mL. After a standing for 24 hours, filter the solution through a glass filter (G3 or G4), if necessary, and standardize the solution by the following method:

Standardization Add exactly 25mL of the prepared cerium (IV) tetraammonium sulfate solution into an iodine flask. Add 20mL of water and 20mL of dilute sulfuric acid, then dissolve 1 g of potassium iodide in the mixture. Immediately titrate the solution with 0.1 mol/L sodium thiosulfate VS. When a pale yellow color, indicating the imminent end point, appears, add 3mL of starch TS. Continue the titration, until the blue color disappears. After a blank determination, calculate the molarity factor.

Note Store protected from light. After a prolonged storage, this solution requires restandardization.

Cerium (IV) Tetraammonium Sulfate, 0.01 mol/L

1000mL of this solution contains 6.326 g of cerium (IV) tetraammonium sulfate dihydrate [$Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$: 632.55].

Preparation Prior to use, dilute 0.1 mol/L cerium (IV) tetraammonium sulfate VS with 0.5 mol/L sulfuric acid VS to make exactly 10 times the initial volume.

Disodium Ethylenediaminetetraacetate, 0.1 mol/L

1000mL of this solution contains 37.224 g of disodium ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 38 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000mL, and standardize the solution by the following method:

Standardization Wash zinc (standard reagent) with diluted hydrochloric acid, water, and then acetone, dry at 110 °C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 1.3 g of the dried zinc, add 20mL of diluted hydrochloric acid and 8 drops of bromine TS, and dissolve it by gentle warming. Eliminate excess of bromine by boiling, and add water to make exactly 200mL. Pipet 25mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate the solution with the prepared disodium ethylenediaminetetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

EachmL of 0.1 mol/L disodium
ethylenediaminetetraacetate VS = 6.539 mg of Zn

Note Store in polyethylene bottles.

Disodium Ethylenediaminetetraacetate, 0.05 mol/L

1000mL of this solution contains 18.612 g of disodium ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 19 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000mL, and standardize the solution by the following method:

Standardization Wash zinc (standard reagent) with diluted hydrochloric acid, water and then acetone, dry at 110 °C for 5 minutes, and allow to cool in a silica gel desiccator. Weigh accurately about 0.8 g of the dried zinc, add 12mL of diluted hydrochloric acid and 5 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200mL. Measure exactly 20mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate the solution with the prepared disodium ethylenediamine-tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

EachmL of 0.05 mol/L disodium
ethylenediaminetetraacetate VS = 3.2695 mg of Zn

Note Store in polyethylene bottles.

Disodium Ethylenediaminetetraacetate, 0.02 mol/L

1000mL of this solution contains 7.445 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2 \cdot Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Dissolve 7.5 g of disodium ethylenediaminetetraacetate dihydrate in water to make 1000mL, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 0.05 mol/L disodium ethylenediamine tetraacetate VS. Wash zinc (standard reagent) with diluted hydrochloric acid, with water, and with acetone, and then, cooled after drying in a silica gel desiccator at 110 °C for 5 minutes. Accurately weigh 0.3 g of the washed zinc, add 5mL of dilute hydrochloric acid and 5 drops of bromine TS.

EachmL of 0.02 mol/L disodium
ethylenediaminetetraacetate VS = 1.3078 mg of Zn

Note Store in polyethylene bottles.

Disodium Ethylenediaminetetraacetate, 0.01 mol/L

1000mL of this solution contains 3.7224 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2 \cdot Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Prior to use, dilute 0.02 mol/L disodium ethylenediaminetetraacetate VS with water to make exactly twice the initial volume.

Disodium Ethylenediaminetetraacetate, 0.001 mol/L

1000mL of this solution contains 0.37224 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2 \cdot Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation Prior to use, dilute 0.01 mol/L disodium ethylenediaminetetraacetate VS with water to make exactly 10 times the initial volume.

Ferric Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (III) Sulfate, 0.1 mol/L.

Ferrous Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (II) Sulfate, 0.1 mol/L.

Ferrous Ammonium Sulfate, 0.02 mol/L

See Ammonium Iron (II) Sulfate, 0.02 mol/L.

Hydrochloric Acid, 2 mol/L

1000mL of this solution contains 72.92 g of hydrochloric acid (HCl: 36.461).

Preparation Dilute 180mL of hydrochloric acid with water to make 1000mL, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 1 mol/L hydrochloric acid VS. For the preparation of sodium carbonate solution, weigh about 2.6 g of sodium carbonate (standard reagent) accurately, and dissolve in 100mL of water.

EachmL of 2 mol/L hydrochloric acid VS
= 105.99 mg of Na_2CO_3

Hydrochloric Acid, 1 mol/L

1000mL of this solution contains 36.461 g of hydrochloric acid (HCl: 36.461).

Preparation Dilute 90mL of hydrochloric acid with water to make 1000mL, and standardize the solution by the following method:

Standardization Accurately weigh about 1.3 g of sodium carbonate (standard reagent), which is previously heated between 500 °C and 650 °C for 40 to 50 minutes and allowed to cool in a silica gel desiccator. Dissolve the dried sodium carbonate in 50mL of water, add 3 drops of methyl red TS and titrate with the prepared hydrochloric acid. When the end-point is approached, boil the content with care, stopper the flask loosely, allow to cool, and continue the titration until the color of the solution changes to persistent orange to orange-red. Calculate the molarity factor.

EachmL of 1 mol/L hydrochloric acid VS
= 52.99 mg of Na_2CO_3

Hydrochloric Acid, 0.5 mol/L

1000mL of this solution contains 18.230 g of hydrochloric acid (HC1: 36.461).

Preparation Dilute 45mL of hydrochloric acid with water to make 1000mL, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 1 mol/L hydrochloric acid VS. For the preparation of sodium carbonate solution, accurately weigh about 0.7 g of sodium carbonate (standard reagent), and dissolve in 50mL of water.

Hydrochloric Acid, 0.2 mol/L

1000mL of this solution contains 7.292 g of hydrochloric acid (HCl: 36.461).

Preparation Dilute 18mL of hydrochloric acid with water to make 1000mL, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 1 mol/L hydrochloric acid VS. For the preparation of sodium carbonate solution, accurately about 0.3 g of sodium carbonate (standard reagent), and dissolve in 30mL of water.

EachmL of 0.2 mol/L hydrochloric acid VS
= 10.599 mg of Na_2CO_3

Hydrochloric Acid, 0.1 mol/L

1000mL of this solution contains 3.6461 g of hydrochloric acid (HCl: 36.461).

Preparation Dilute 9.0mL of hydrochloric acid with water to make 1000mL, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 1 mol/L hydrochloric acid VS. For the preparation of sodium carbonate solution, accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30mL of water.

EachmL of 0.1 mol/L hydrochloric acid VS
= 5.299 mg of Na_2CO_3

Hydrochloric Acid, 0.05 mol/L

1000mL of this solution contains 1.8230 g of hydrochloric acid (HCl: 36.461).

Preparation Prior to use, dilute 0.1 mol/L hydrochloric acid VS with water to make exactly 5 times the initial volume.

Hydrochloric Acid, 0.02 mol/L

1000mL of this solution contains 0.7292 g of hydrochloric acid (HCl: 36.461).

Preparation Prior to use, dilute 0.1 mol/L hydrochloric acid VS with water to make exactly 5 times the initial volume.

Hydrochloric Acid, 0.01 mol/ L

1000mL of this solution contains 0.36461 g of hydrochloric acid (HCl:36.461).

Preparation Prior to use, dilute 0.1 mol/L hydrochloric acid VS with water to make exactly 10 times the initial volume.

Hydrochloric Acid, 0.001 mol/L

1000mL of this solution contains 0.036461 g of hydrochloric acid (HCl: 36.461).

Preparation Prior to use, dilute 0.1 mol/L hydrochloric acid VS with water to make exactly 100 times the initial volume.

Iodine, 0.05 mol/L

1000mL of this solution contains 12.690 g of iodine (I: 126.90).

Preparation Dissolve 13 g of iodine in 100mL of a solution of potassium iodide (2 in 5), add 1mL of diluted hydrochloric acid and water to make 1000mL, and standardize the solution as follows:

Standardization Accurately mass about 0.15 g of arsenic trioxide (standard reagent), which is previously powdered and dried at 105 °C for 3 to 4 hours and allowed to cool in a silica gel desiccator. Dissolve weighed arsenic trioxide in 20mL of a solution of sodium hydroxide (1 in 25), if necessary, by warming. Add 40mL of water, 2 drops of methyl orange TS and then diluted hydrochloric acid until the solution acquires a pale red color, and add subsequently 2 g of sodium bicarbonate, 50mL of water and 3mL of starch TS. Titrate slowly with the prepared iodine solution until a persistent blue color appears. Calculate the molarity factor.

Each mL of 0.05 mol/L iodine VS
= 4.946 mg of As_2O_3

Note Store protected from light. After a prolonged storage, this solution requires restandardization.

Iodine, 0.01 mol/L

1000mL of this solution contains 2.5381 g of iodine (I: 126.90).

Preparation Prior to use, dilute 0.05 mol/L iodine VS with water to make exactly 5 times the initial volume.

Iodine, 0.005 mol/L

1000mL of this solution contains 1.2690 g of iodine (I: 126.90).

Preparation Prior to use, dilute 0.05 mol/L iodine VS with water to make exactly 10 times the initial volume.

Iodine, 0.002 mol/L

1000mL of this solution contains 0.5076 g of iodine (I: 126.90).

Preparation Prior to use, dilute 0.05 mol/L iodine VS with water to make exactly 25 times the initial volume.

Magnesium Chloride, 0.05 mol/L

1000mL of this solution 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 1000mL, and standardize the solution by the following method:

Standardization Add 50mL of water, 3mL of pH 10.7 ammonia-ammonium chloride buffer solution to exactly 25mL of the prepared magnesium chloride solution. Add 0.04 g of eriochrome black T-sodium chloride reagent to the mixture, and titrate with 0.05 mol/L disodium ethylenediaminetetraacetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

Magnesium Chloride, 0.01 mol/L

1000mL of this solution contains 2.0330 g of magnesium chloride hexahydrate ($MgC1_2 \cdot 6H_2O$: 203.30).

Preparation Prior to use, dilute 0.05 mol/L magnesium chloride VS with water to make exactly 5 times the initial volume.

Mercuric Acetate, 0.05 mol/L

See Mercury (II) Acetate, 0.05 mol/L.

Mercuric Acetate, 0.005 mol/L

See Mercury (II) Acetate, 0.005 mol/L.

Mercury (II) Acetate, 0.05 mol/L

1000mL of this solution contains 15.934 g of mercury (II) acetate [$Hg(CH_3COO)_2$: 318.68].

Preparation Dissolve 16 g of mercury (II) acetate in 5mL of glacial acetic acid and water to make 1000mL, and standardize the solution by the following method:

Standardization Accurately weigh about 5.8 g of sodium chloride (standard reagent) which previously dried between 500 °C and 650 °C for 40 to 50 minutes, and allowed to cool in a silica gel desiccator. Dissolve the weighed sodium chloride in water to make exactly 1000mL. Measure exactly 20mL of this solution, add 1 drop of bromophenol blue TS, and dilute nitric acid drop-wise until a yellow color appears, and add a further 5mL of diluted nitric acid, 100mL of methanol and 1mL of diphenylcarbazone TS. With a thorough agitation, titrate the solution with the prepared mercuric acetate solution until the pale yellow color changes to red-purple, and calculate the molarity factor.

Each mL of 0.05 mol/L mercuric acetate VS
= 5.844 mg of NaCl

Mercury (II) Acetate, 5 mmol/L

1000mL of this solution contains 1.5934 g of mercury (II) acetate [$Hg(CH_3COO)_2$: 318.68].

Preparation Dissolve 1.6 g of mercury (II) acetate in 60mL of diluted nitric acid (1 in 10), add water to make 1000mL, and standardize the solution as follows:

Standardization Accurately weigh about 0.58 g of sodium chloride (standard reagent) which is previously dried between 500 °C and 650 °C for 40 to 50 minutes, and allowed to cool in a silica gel desiccator. Dissolve

the weighed sodium chloride in water to make exactly 1000mL. Measure exactly 20mL of this solution, add 1 drop of bromophenol blue TS and diluted nitric acid drop-wise until a yellow color develops, and add 5mL of diluted nitric acid, 100mL of methanol, and 1mL of diphenylcarbazone TS. With a thorough agitation, titrate the solution with the prepared mercuric acetate solution until the pale yellow color changes to red-purple, and calculate the molarity factor

EachmL of 0.005 mol/L mercuric acetate VS
= 0.5844 mg of NaCl

Oxalic Acid, 0.05 mol/L

1000mL of this solution contains 6.303 g of oxalic acid ($C_2H_2O_2 \cdot 2H_2O$: 126.07).

Preparation Dissolve 6.3 g of oxalic acid in water to make 1000mL, and standardize the solution by the following method:

Standardization Add exactly 25mL of the prepared oxalic acid solution to a 500mL conical flask. Add 200mL of diluted sulfuric acid (1 in 20), which is previously boiled for 10 to 15 minutes and then cooled to 27 ± 3 °C, to the flask. Add freshly standardized 0.02 mol/L potassium permanganate VS to a burette. Add quickly 22mL of the 0.02 mol/L potassium permanganate VS to the oxalic acid solution from the burette with a gentle agitation, and allow to stand until the red color of the mixture disappears. Heat the solution between 55 °C and 60 °C, and complete the titration by adding 0.02 mol/L potassium permanganate VS until a faint red color persists for 30 seconds. Add the last 0.5 to 1mL drop-wise fashion so that the next drop is added after the solution is decolorized. Calculate the molarity factor.

Note Store protected from light.

Oxalic Acid, 5 mmol/L

1000mL of this solution contains 0.6303 g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$: 126.07).

Preparation Priot to use, dilute 0.05 mol/L oxalic acid VS with water to make exactly 10 times the initial volume.

Perchloric Acid, 0.1 mol/L

1000mL of this solution contains 10.046 g of perchloric acid ($HClO_4$:100.46).

Preparation While maintaining the termperature at about 20 °C, slowly add 8.7mL of perchloric acid to 1000mL of acetic acid (100). Allow the mixture to stand for about 1 hour. Quickly determine the water content (A in w/v %) with 3.0mL of the mixture. To the mixture, add slowly (A - 0.03)×52.2mL of acetic anhydride with an agitation at about 20 °C. After a standing for 24 hours, standardize the solution by the following method

Standardization Accurately weigh about 0.5 g of potassium hydrogen phthalate (standard reagent) which is previously dried at 105 °C for 4 hours, and allowed to cool in a silica gel desiccator. Dissolve the weighed potassium hydrogen phthalate in 80mL of acetic acid (100), and add 3 drops of crystal violet TS. Titrate the solution with the prepared perchloric acid solution until a blue color appears. After a blank determination, calculate the molarity factor.

EachmL of 0.1 mol/L perchloric acid VS
= 20.422 mg of $KHC_6H_4(COO)_2$

Note Store protected from moisture.

Perchloric Acid, 0.05 mol/L

1000mL of this solution contains 5.023 g of perchloric acid ($HClO_4$: 100.46).

Preparation Prior to use, dilute 0.1 mol/L perchloric acid VS with glacial acetic acid for nonaqueous titration to make exactly twice the initial volume. Quickly determine the water content (A, w/v %) with 8.0mL of glacial acetic acid. If A is more than 0.03, add (A - 0.03)×52.2mL of acetic anhydride to 1000mL of glacial acetic acid for nonaqueous titration, and use it for the preparation.

Perchloric Acid, 0.02 mol/L

1000mL of this solution contains 2.0092 g of perchloric acid ($HClO_4$: 100.46).

Preparation Prior to use, dilute 0.1mol/L perchloric acid VS with glacial acetic acid for nonaqueous titration to make exactly 5 times the initial volume. Quickly determine the water content (A, w/v %) with 8.0mL of glacial acetic acid. If A is more than 0.03, add (A - 0.03)×52.2mL of acetic anhydride to 1000mL of glacial acetic acid for nonaqueous titration, and use it for the preparation.

Perchloric Acid-Dioxane, 0.1 mol/L

1000mL of this solution contains 10.046 g of perchloric acid ($HClO_4$: 100.46).

Preparation Dilute 8.5mL of perchloric acid with 1,4-dioxane to make 1000mL, and standardize the solution by the following method:

Standardization Accurately weigh about 0.5 g of potassium biphthalate (standard reagent) which is previously dried at 105 °C for 4 hours, and allowed to cool in a silica gel desiccator. Dissolve the weighed potassium biphthalate in 80mL of glacial acetic acid for nonaqueous titration, and add 3 drops of crystal violet TS. Titrate the solution with the prepared perchloric acid-dioxane solution until a blue color appears. After a blank determination, calculate the molarity factor.

EachmL of 0.1 mol/L perchloric acid-dioxane VS
= 20.422 mg of $KHC_6H_4(COO)_2$

Note Store in a cold place, protected from moisture.

Perchloric Acid-Dioxane, 0.05 mol/L

1000mL of this solution contains 5.023 g of perchloric acid ($HClO_4$:100.46).

Preparation Prior to use, dilute 0.1 mol/L perchloric acid-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

Perchloric Acid-Dioxane, 4 mmol/L

1000mL of this solution contains 0.4018 g of perchloric acid ($HClO_4$: 100.46).

Preparation Prior to use, dilute 0.1mol/L perchloric acid-dioxane VS with 1,4-dioxane to make exactly 25 times the initial volume.

Potassium Bichromate, 1/60 mol/L

1000mL of this solution contains 4.903 g of potassium bichromate ($K_2Cr_2O_7$: 294.18).

Preparation Accurately weigh about 4.903 g of potassium bichromate (standard reagent) which is previously powdered, dried between 100 °C and 110 °C for 3 to 4 hours, and allowed to cool in a silica gel desiccator. Dissolve the weighed potassium bichromate in water to make exactly 1000mL, and calculate the molarity factor.

Potassium Bromate, 1/60 mol/L

1000mL of this solution contains 2.7833 g of potassium bromate ($KBrO_3$: 167.00).

Preparation Dissolve 2.8 g of potassium bromate in water to make 1000mL, and standardize the solution by the following method

Standardization Add exactly 25mL of the prepared potassium bromate solution into an iodine flask. Add 2 g of potassium iodide and 5mL of dilute sulfuric acid, stopper the flask, and allow the solution to stand for 5 minutes. Add 100mL of water, and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS. When a pale yellow color, indicating the imminent end point, appears, add 3mL of starch TS. Continue the titration, until the blue color disappears. After a blank determination, calculate the molarity factor.

Potassium Ferricyanide, 0.1 mol/L

See Potassium Hexacyanoferrate (III), 0.1 mol/L.

Potassium Ferricyanide, 0.05 mol/L

See Potassium Hexacyanoferrate (III), 0.05 mol/L.

Potassium Hexacyanoferrate(III), 0.1 mol/L

1000mL of this solution 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 1000mL, and standardize the solution by the following method:

Standardization Add exactly 25mL of the prepared potassium hexacuanoferrate (III) solution into an iodine flask. Add 2 g of potassium iodide and 10mL of dilute hydrochloric acid, stopper the flask, and allow to stand for 15 minutes. Add 15mL of zinc sulfate TS, and titrate the generated iodine with 0.1 mol/L sodium thiosulfate VS. When a pale yellow color, indicating the imminent end point, appears, add 3mL of starch TS. Continue the titration, until the blue color disappears. After a blank determination, calculate the molarity factor.

Note Store protected from Light. After a prolonged storage, this solution requires restandardization.

Potassium Hexacyanoferrate (III), 0.05 mol/L

1000mL of this solution contains 16.462 g of potassium hexacyanoferrate (III) [$K_3Fe(CN)_6$: 329.25].

Preparation Prior to use, dilute 0.1 mol/L potassium ferricyanide VS with water to make exactly twice the initial volume.

Potassium Hydroxide, 1 mol/L

1000mL of this solution contains 56.11 g of potassium hydroxide (KOH: 56.11).

Preparation Dissolve 65 g of potassium hydroxide in 950mL of water. Add a freshly prepared, saturated solution of barium hydroxide until precipitate is no longer produced. With a thorough agitation, allow it to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or 04), and standardize the solution by the following method:

Standardization Accurately weigh about 2.5 g of amidosulfuric acid (standard reagent) which is previously dried in a silica gel desiccator (in vacuum) for about 48 hours. Dissolve the weighed amidosulfuric acid in 25mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate the solution with the prepared potassium hydroxide solution until a green color appears. Calculate the molarity factor.

EachmL of 1 mol/L potassium hydroxide VS
= 97.09 mg of HOSO2NH2

Note Store in tightly stoppered bottles or in containers having a carbon dioxide-absorbing tube (soda-lime). After a prolonged storage, this solution requires restandardization.

Potassium Hydroxide, 0.5mol/L

1000mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 32 g of potassium hydroxide, proceed similarly to preparation for 1 mol/L potassium hydroxide VS, and standardize the solution by the following method:

Standardization Proceed similarly to 1 mol/L potassium hydroxide VS. For the preparation of amidosulfuric acid solution, accurately weigh about 1.3 g of amidosulfuric acid (standard reagent).

EachmL 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 VS. After a prolonged storage, this solution requires restandardization.

Potassium Hydroxide, 0.1 mol/L

1000mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 6.5 g of potassium hydroxide, proceed similarly to preparation for 1 mol/L potassium hydroxide VS, and standardize the solution by the following method:

Standardization Proceed similarly to 1 mol/L potassium hydroxide VS. For the preparation of amidosulfuric acid solution, accurately weigh about 0.25 g of amidosulfuric acid (standard reagent).

EachmL of 0.1 mol/L potassium hydroxide VS
= 9.709 mg of $HOSO_2NH_4$

Note Store as directed under 1 mol/L potassium hydroxide VS. After a prolonged storage, this solution requires restandardization.

Potassium Hydroxide-Ethanol, 0.5 mol/L

1000mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation Dissolve 35 g of potassium hydroxide in 20mL of water, and add aldehyde-free ethanol to make 1000mL. Allow the solution to stand for 24 hours in a tightly stoppered bottle. Then quickly decant the supernatant liquid, and standardize the solution by the following method:

Standardization Add 50mL of water and 2 drops of phenolphthalein TS to exactly 25mL of 0.25 mol/L sulfuric acid VS. Titrate the solution with the prepared potassium hydroxide-ethanol solution until pale red color appears. Calculate the molarity factor.

Note Store in tightly stoppered bottles, protected from light. Standardize just prior to use.

Potassium Hydroxide-Ethanol, 0.1 mol/L

1000mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 7 g of potassium hydroxide, proceed similarly to preparation for 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 0.5 mol/L potassium hydroxide-ethanol VS. For the measurement of sulfuric acid, use exactly 25mL of 0.05 mol/L sulfuric acid VS.

Note Store as directed under 0.5 mol/L potassium hydroxide-ethanol VS. Standardize before use.

Potassium Hydroxide-Ethanol, 0.05 mol/L

1000mL of this solution contains 2.8055 g of potassium hydroxide (KOH: 56.11).

Preparation Weigh 3.5 g of potassium hydroxide, proceed similarly to preparation for 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution by the following method:

Standardization Proceed similarly to standardization for 0.5 mol/L potassium hydroxide-ethanol VS. For the measurement of sulfuric acid, use exactly 25mL of 0.025 mol/L sulfuric acid VS.

Note Store as directed under 0.5 mol/L potassium hydroxide-ethanol VS. Standardize before use.

Potassium Hydroxide-Propanol-Benzene, 0.1 mol/L

1000mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation Wash 7 g of potassium hydroxide with 50mL of 1-propanol, then dissolve it in 250mL of 1-propanol with shaking, add dehydrated benzene to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.26 g of benzoic acid, previously dried in a desiccator (silica gel) for 3 hours, dissolve it in 50mL of dimethylformamide, add 10 drops of metanil yellow TS, and titrate the solution with the prepared potassium hydroxide-propanol-benzene solution until a blue-purple color is produced. Calculate the molarity factor.

EachmL of 0.1 mol/L potassium hydroxide-propanol-benzene VS = 12.212 mg of C_6H_5COOH

Note Store in tightly stoppered bottles, protected from light.

Potassium Iodate, 0.05 mol/L

1000mL of this solution contains 10.700 g of potassium iodate (KIO_3: 214.00).

Preparation Weigh accurately about 10.700 g of potassium iodate (standard reagent), previously dried between 120 °C and 140 °C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000mL. Calculate the molarity factor.

Potassium Iodate, 1/1200 mol/L

1000mL of this solution contains 0.17833 g of potassium iodate (KIO_3: 214.00).

Preparation Weigh accurately about 0.17833 g of potassium iodate, previously dried between 120 °C and 140 °C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000mL. Calculate the molarity factor.

Potassium Permanganate, 0.02 mol/L

1000mL of this solution contains 3.1607 g of potassium permanganate ($KMnO_4$: 158.03).

Preparation Dissolve 3.2 g of potassium permanganate in water to make 1000mL, and boil the solution for 15 minutes. Allow the solution to stand for at least 48 hours in a tightly stoppered flask, and filter it through a glass filter (G3 or G4). Standardize the solution as follows:

Standardization Weigh accurately about 0.3 g of sodium oxalate (standard reagent), previously dried between 150 °C and 200 °C for 1 to 1.5 hours and allowed to cool in a desiccator (silica gel), transfer it to a 500mL conical flask, dissolve in 30mL of water, add 250mL of diluted sulfuric acid (1 in 20), and warm the

mixture between 30 °C and 35 °C. Transfer the prepared potassium permanganate solution to a buret, add quickly 40mL of the solution under gentle stirring from the buret, and allow to stand until the red color of the mixture disappears. Warm the solution between 55 °C and 60 °C, and complete the titration by adding the potassium permanganate solution until a faint red color persists for 30 seconds. Add the last 0.5 to 1mL dropwise before the end point, being particularly careful to allow the solution to be decolorized before the next drop is added. Calculate the molarity factor.

Each mL of 0.02 mol/L potassium permanganate VS
= 6.700 mg of $Na_2C_2O_4$

Note Store protected from light. This solution, if stored for a long period, should be restandardized.

Potassium Permanganate, 2 mmol/L

1000mL of this solution contains 0.31607 g of potassium permanganate ($KMnO_4$: 158.03).

Preparation Before use, dilute 0.02 mol/L potassium permanganate VS with water to make exactly 10 times the initial volume.

Silver Nitrate, 0.1 mol/L

1000mL of this solution contains 16.987 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Dissolve 17.0 g of silver nitrate in water to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.15 g of sodium chloride (standard reagent), previously dried between 500 °C and 650 °C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel), dissolve it in 50mL of water, add 3 drops of fluorescein sodium TS, and titrate under vigorous shaking with the prepared silver nitrate solution until the color of the solution changes from yellow-green through yellow to yellow-orange. Calculate the molarity factor.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Note Store protected from light.

Silver Nitrate, 0.02 mol/L

1000mL of this solution contains 3.3974 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 5 times the initial volume.

Silver Nitrate, 0.01 mol/L

1000mL of this solution contains 1.6987 g of silver nitrate ($AgNO_3$:169.87).

Preparation Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 10 times the initial volume.

Silver Nitrate, 0.005 mol/L

1000mL of this solution contains 0.8494 g of silver nitrate ($AgNO_3$:169.87).

Preparation Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 20 times the initial volume.

Silver Nitrate, 1 mmol/L

1000mL of this solution contains 0.16987 g of silver nitrate ($AgNO_3$: 169.87).

Preparation Dilute 0.1 mol/L silver nitrate VS with water to make exactly 100 times of the initial volume before use.

Sodium Acetate, 0.1 mol/L

1000mL of this solution contains 8.203 g of sodium acetate (CH_3COONa: 82.03).

Preparation Dissolve 8.20 g of anhydrous sodium acetate in glacial acetic acid to make 1000mL, and standardize the solution as follows:

Standardization Pipet 25mL of the prepared sodium acetate solution, add 50mL of glacial acetic acid and 1mL of *p*-naphtholbenzeine TS, and titrate with 0.1 mol/L perchloric acid VS until a yellow-brown color changes through yellow to green. Perform a blank determination. Calculate the molarity factor.

Sodium Hydroxide, 1 mol/L

1000mL of this solution contains 39.997 g of sodium hydroxide (NaOH: 39.997).

Preparation Dissolve 42 g of sodium hydroxide in 950mL of water. Add a freshly prepared, saturated solution of barium hydroxide until no more precipitate is produced. Mix well the mixture, and allow to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

Standardization Weigh accurately about 2.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate the solution with the prepared sodium hydroxide solution until it acquires a green color. Calculate the molarity 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 provided with a carbon dioxide-absorbing tube (soda lime). This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.5 mol/L

1000mL of this solution contains 19.999g of sodium hydroxide (NaOH: 39.997).

Preparation Weigh 22 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:
Standardization Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 1.3 g of amidosulfuric acid (standard reagent).

EachmL of 0.5 mol/L sodium hydroxide VS
= 48.55 mg of $HOSO_2NH_2$

Note Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.2 mol/L

1000mL of this solution contains 7.999 g of sodium hydroxide (NaOH: 39.997).
Preparation Weigh 9 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:
Standardization Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.5 g of amidosulfuric acid (standard reagent).

EachmL of 0.2 mol/L sodium hydroxide VS
=19.419 mg of $HOSO_2NH_2$

Note Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.1 mol/L

1000mL of this solution contains 3.9997 g of sodium hydroxide (NaOH: 39.997).
Preparation Weigh 4.5 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows.
Standardization Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.25 g of amidosulfuric acid (standard reagent).

EachmL of 0.1 mol/L sodium hydroxide VS
= 9.709 mg of $HOSO_2NH_2$

Note Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.05 mol/L

1000mL of this solution contains 1.9999 g of sodium hydroxide (NaOH: 39.997).
Preparation Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly twice the initial volume.

Sodium Hydroxide, 0.02 mol/L

1000mL of this solution contains 0.7999 g of sodium hydroxide (NaOH: 39.997).
Preparation Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Sodium Hydroxide, 0.01 mol/L

1000mL of this solution contains 0.39997 g of sodium hydroxide (NaOH: 39.997).
Preparation Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

Sodium Lauryl Sulfate, 0.01 mol/L

1000mL of this solution 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 1000mL, and standardize the solution as follows:
Standardization Weigh accurately about 0.3 g of papaverine for assay, previously dried, and dissolve in water to make exactly 100mL. Pipet 10mL of this solution into a glass-stoppered conical flask, add 5mL each of water and dilute sulfuric acid and 60mL of dichloromethane, then add 5 to 6 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate, while vigorously shaking, with 0.01 mol/L sodium lauryl sulfate VS, using a buret with a minimum graduation of 0.02mL. End point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of 0.01 mol/L sodium lauryl sulfate VS, vigorous shaking and standing for a while.

EachmL of 0.01 mol/L sodium lauryl sulfate VS
= 3.7585 mg of $C_{20}H_{21}NO_4 \cdot HC1$

Sodium Methoxide, 0.5 mol/L (in methanol)

1000mL of this solution contains 27.01 g of sodium methoxide (CH_3ONa: 54.02).
Preparation Weigh 11.5 g of freshly cut sodium metal, and cut into small cubes. Place about 0.5mL of anhydrous methanol in a round-bottom, 250mL flask equipped with a ground-glass joint, add 1 cube of the sodium metal, and, when the reaction has ceased, add the remaining sodium metal to the flask. Connect a water-jacketed condenser to the flask, and slowly add 250mL of anhydrous methanol, in small portions, through the top of the condenser. Regulate the addition of the methanol so that the vapors are condensed and do not escape through the top of the condenser. After addition of the methanol is complete, connect a drying tube to the top of the condenser, and allow the solution to cool. Transfer the solution to a 1000mL volumetric flask, dilute with anhydrous methanol to volume, and mix. Standardize the solution as follows.
Standardization Accurately measure about 20mL of freshly standardized 1 mol/L hydrochloric acid VS into a 250mL conical flask, add 0.25mL of phenolphthalein

TS, and titrate with the sodium methoxide solution to the first appearance of a permanent pink color.

Sodium Methoxide, 0.1 mol/L

1000mL of this solution contains 5.402 g of sodium methoxide (CH_3ONa: 54.02).

Preparation Add little by little, 2.5 g of freshly cut metallic sodium pieces to 150mL of methanol cooled in ice-water. After the metal has dissolved, add benzene to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.3 g of benzoic acid, previously dried for 24 hours in a desiccator (silica gel), dissolve it in 80mL of dimethylformamide, and add 3 drops of thymol blue-dimethylformamide TS. Titrate the solution with the prepared sodium methoxide solution until a blue color appears. Perform a blank determination, and make any necessasary correction. Calculate the molarity factor.

EachmL of 0.1 mol/L sodium methoxide VS
=12.212 mg of C_6H_5COCH

Note Store in a cold place, protected from moisture. Standardize before use.

Sodium Methoxide-Dioxane, 0.1 mol/L

1000mL of this solution contains 5.402 g of sodium methoxide (CH_3ONa:54.02).

Preparation Add little by little 2.5 g of freshly cut metallic sodium pieces to 150mL of methanol cooled in ice-water. After the metal has dissolved, add 1,4-dioxane to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 80mL of dimethylformamide, and add 3 drops of thymol blue-dimethylformamide TS. Titrate the solution with the prepared sodium methoxide-dioxane solution until a blue color appears. Perform a blank determination, and make any necerssary correction. Calculate the molarity factor.

EachmL of 0.1 mol/L sodium methoxide-dioxane VS
= 12.212 mg of C_6H_5COOH

Note Store in a cold place, protected from moisture. Standardize before use.

Sodium Nitrite, 0.1 mol/L

1000mL of this solution contains 6.900 g of sodium nitrite ($NaNO_2$: 69.00).

Preparation. Dissolve 7.2 g of sodium nitrite in water to make 1000mL, and standardize the solution as follows:

Standardization. Weigh accurately about 0.44 g of sulfanilamide for titration of diazotization, previously dried at 105 °C for 3 hours and allowed to cool in a desiccator (silica gel), dissolve in 10mL of hydrochloric acid, 40mL of water and 10mL of a solution of potassium bromide (3 in 10), cool below 159C, and titrate with the prepared sodium nitrite solution as directed in the potentiometric titration or amperometric titration under Endpoint Detection Methods in Titrimetry. Calculate the molarity factor.

EachmL of 0.1 mol/L sodium nitrite VS
= 17.22 mg of $H_2NC_6H_4SO_2NH_2$

Note: Store protected from light. This solution, if stored for a long period, should be restandadized.

Sodium Oxalate, 0.005 mol/L

1000mL of this solution contains 0.6700 g of sodium oxalate ($Na_2C_2O_4$: 134.00).

Preparation Weigh accurately about 0.6700 g of sodium oxalate (standard reagent), previously dried between 150 °C and 200 °C for 2 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000mL, and calculate the molarity factor.

Sodium Tetraphenylborate, 0.02 mol/L

1000mL of this solution contains 6.844 g of sodium tetraphenylborate [NaB(C6H5)4: 342.22].

Preparation Dissolve 7.0 g of sodium tetraphenylborate in water to make 1000mL, and standardize the solution as follows:

Standardization Weigh 0.5 g of potassium hydrogen phthalate (standard reagent), dissolve it in 100mL of water, add 2mL of acetic acid, and warm to 50 °C in a water-bath. Add slowly 50mL of the prepared sodium tetraphenylborate solution under stirring from a buret, then cool the mixture quickly, and allow to stand for 1 hour at room temperature. Transfer the precipitate to a tared glass filter (G4), wash with three 5mL volumes of potassium tetraphenylborate TS, dry at 105 °C for 1 hour, and weigh accurately the glass filter. Calculate the molarity factor from the mass of potassium tetraphenylborate [$KB(C_6H_5)_4$: 358.33].

EachmL of 0.02 mol/L sodium tetraphenylborate VS
= 7.167 mg of $KB(C_6H_5)_4$

Note Prepare before use.

Sodium Tetraphenylboron, 0.02 mol/L

See Sodium Tetraphenylborate, 0.02 mol/L.

Sodium Thiosulfate, 0.1 mol/L

1000mL of this solution contains 24.819 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$: 248.19).

Preparation Dissolve 25 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000mL, allow to stand for 24 hours, and standardize the solution as follows:

Standardization Weigh accurately about 0.1 g of potassium iodate (standard reagent), previously dried between 120 °C and 140 °C for 1.5 to 2 hours and al-

lowed to cool in a desiccator (silica gel), and transfer to an iodine flask. Dissolve it in 25mL of water, add 2 g of potassium iodide and 10mL of dilute sulfuric acid, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 100mL of water, and titrate the liberated iodine with the prepared sodium thiosulfate solution. When the solution assumes a pale yellow color as the end point is approached, add 3mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

EachmL of 0.1 mol/L sodium thiosulfate VS
= 3.5667 mg of KIO_3

Note This solution, if stored for a long period, should be restandardized.

Sodium Thiosulfate, 0.05 mol/L

1000mL of this solution contains 12.409 g of sodium thiosulfate ($Na_2S_2O_3.5H_2O$: 248.19).

Preparation Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 2 times the initial volume.

Sodium Thiosulfate, 0.02 mol/L

1000mL of this solution contains 4.964 g of sodium thiosulfate ($Na_2S_2O_3.5H_2O$: 248.19).

Preparation Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Sodium Thiosulfate, 0.01 mol/L

1000mL of this solution contains 2.4819 g of sodium thiosulfate ($Na_2S_2O_3.5H_2O$: 248.19).

Preparation Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

Sodium Thiosulfate, 0.005 mol/L

1000mL of this solution contains 1.2409 g of sodium thiosulfate (Na2S2O3·5H2O: 248.19).

Preparation Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 20 times the initial volume.

Sulfuric Acid, 0.5 mol/L

1000mL of this solution contains 49.04 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add slowly, under stirring, 30mL of sulfuric acid to 1000mL of water, allow to cool, and standardize the solution as follows:

Standardization Weigh accurately about 1.3 g of sodium carbonate (standard reagent), previously heated between 500 °C and 650 °C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50mL of water, add 3 drops of methyl red TS, and titrate the solution with the prepared sulfuric acid. When the end point is approached, boil the solution carefully, stopper the flask loosely, allow to cool, and continue the titration, until the color of the solution changes to persistent orange to orange-red. Calculate the molarity factor.

EachmL of 0.5 mol/L sulfuric acid VS
= 52.99 mg of Na_2CO_3

Sulfuric Acid, 0.25mol/L

1000mL of this solution contains 24.520 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add slowly, under stirring, 15mL of sulfuric acid to 1000mL of water, allow to cool, and standardize the solution as follows:

Standardization Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.7 g of sodium carbonate (standard reagent), and dissolve in 50mL of water.

EachmL of 0.25 mol/L sulfuric acid VS
= 26.497 mg of Na_2CO_3

Sulfuric Acid, 0.1 mol/L

1000mL of this solution contains 9.808 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add slowly, under stirring, 6mL of sulfuric acid to 1000mL of water, allow to cool, and standardize the solution as follows:

Standardization Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.3 g of sodium carbonate (standard reagent), and dissolve in 50mL of water.

EachmL of 0.1 mol/L sulfuric acid VS
= 10.599 mg of Na_2CO_3

Sulfuric Acid, 0.05 mol/L

1000mL of this solution contains 4.904 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Add slowly, under stirring, 3mL of sulfuric acid to 1000mL of water, allow to cool, and standardize the solution as follows:

Standardization Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30mL of water.

EachmL of 0.05 mol/L sulfuric acid VS
= 5.299 mg of Na_2CO_3

Sulfuric Acid, 0.025 mol/L

1000mL of this solution contains 2.4520 g of sulfuric acid (H_2SO_4:98.08).

Preparation Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly twice the initial volume.

Sulfuric Acid, 0.01 mol/L

1000mL of this solution contains 0.9808 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 5 times the initial volume.

Sulfuric Acid, 0.005 mol/L

1000mL of this solution contains 0.4904 g of sulfuric acid (H_2SO_4:98.08).

Preparation Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 10 times the initial volume.

Sulfuric Acid, 0.0005 mol/L

1000mL of this solution contains 0.04904 g of sulfuric acid (H_2SO_4: 98.08).

Preparation Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 100 times the initial volume.

Tetrabutyl Ammonium Hydroxide, 0.1 mol/L

1000mL of this solution contains 25.948 g of tetrabutyl ammonium hydroxide [$(C_4H_9)_4NOH$: 259.48]

Preparation Before use, dilute a volume of 10 % tetrabutyl ammonium hydroxide-methanol TS, equivalent to 26.0 g of tetrabutyl ammonium hydroxide, with isopropanol to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 50mL of acetone, and titrate the solution with the prepared tetrabutyl ammonium hydroxide solution (electrometric titration). Perform a blank determination in the same manner.

EachmL of 0.1 mol/L tetrabutyl ammonium hydroxide VS = 12.212 mg of C_6H_5COOH

Note Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethyl Ammonium Hydroxide, 0.2 mol/L

1000mL of this solution contains 18.231 g of tetramethyl ammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Before use, dilute a volume of tetramethyl ammonium hydroxide-methanol TS, equivalent to 18.4 g of tetramethyl ammonium hydroxide, in water to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.6 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 90mL of dimethylformamide, add 3 drops of thymol blue-dimethylformamide TS, and titrate the solution with the prepared tetramethyl ammonium hydroxide solution until a blue color is produced. Perform a blank determination in the same manner. Calculate the molarity factor.

EachmL of 0.2 mol/L tetramethyl ammonium hydroxide VS = 24.425 mg of C_6H_5COOH

Note Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethyl Ammonium Hydroxide, 0.1 mol/L

1000mL of this solution contains 9.115 g of tetramethyl ammonium hydroxide [$(CH_3)_4NOH$: 91.15).

Preparation Before use, dilute a volume of tetramethyl ammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethyl ammonium hydroxide, in water to make 1000mL, and standardize the solution as follows:

Standardization Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica get) for 24 hours, dissolve it in 90mL of dimethylformamide, add 3 drops of thymol blue-dimethylformamide TS, and titrate the solution with the prepared tetramethylammonium hydroxide solution until a blue color is produced. Perform a blank determination. Calculate the molarity factor.

EachmL of 0.1 mol/L tetramethyl ammonium hydroxide VS = 12.212 mg of C_6H_5COOH

Note Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethyl Ammonium Hydroxide, 0.02 mol/L

1000mL of this solution contains 1.8231 g of tetramethyl ammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Before use, dilute 0.1 mol/L tetramethyl ammonium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Tetramethyl Ammonium Hydroxide-Methanol, 0.1 mol/L

1000mL of this solution contains 9.115 g of tetramethyl ammonium hydroxide [$(CH_3)_4NOH$: 91.15].

Preparation Before use, dilute a volume of tetramethyl ammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethyl ammonium hydroxide, with methanol to make 1000mL, and standardize the solution as follows:

Standardization Proceed as directed for standardization under 0.1 mol/L tetramethyl ammonium hydroxide VS.

Note Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Titanium (III) Chloride, 0.1 mol/L

1000mL of this solution contains 15.424 g of titanium (III) chloride solution ($TiCl_3$: 154.24).

Preparation Add 75mL of hydrochloric acid to 75mL of titanium (III) chloride solution, and dilute with freshly boiled and cooled water to make 1000mL. Transfer the solution into a buret provided with a reservoir protected from light, replace the air with hydrogen, and allow to stand for 48 hours. Before use, standardize the solution as follows:

Standardization Weigh 3 g of ammonium iron (II) sulfate hexahydrate in a wide-mouthed, 500mL conical

flask. Passing carbon dioxide through the flask, dissolve it in 50mL of freshly boiled and cooled water, and add 25mL of diluted sulfuric acid (27 in 100). Passing carbon dioxide through the flask, and rapidly add exactly 40mL of 0.02 mol/L potassium permanganate VS to the mixture. Titrate with the prepared titanium (III) chloride until the calculated end point is approached, then add 5 g of ammonium thiocyanate immediately, and continue the titration with the prepared 0.1 mol/L titanium (III) chloride until the color of the solution disappears. Perform a blank determination. Calculate the molarity factor.
Note Store after the air has been displaced with hydrogen.

Titanium Trichloride, 0.1 mol/L
See Titanium (III) Chloride, 0.1 mol/L.

Zinc, 0.1 mol/L
1000mL of this solution contains 6.539 g of zinc (Zn: 65.39).
Preparation To 6.539 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and then acetone, and cooled in a desiccator (silica gel) after drying at 110 °C for 5 minutes, add 80mL of dilute hydrochloric acid and 2.5mL of bromine TS, dissolve by gentle warming, evaporate excess bromine by boiling, and add water to make exactly 1000mL.

Zinc Acetate, 0.05 mol/L
1000mL of this solution contains 10.975 g of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$: 219.51].
Preparation Dissolve 11.1 g of zinc acetate dihydrate in 40mL of water and 4mL of dilute acetic acid, add water to make 1000mL, and standardize the solution as follows:
Standardization Measure exactly 20mL of 0.05 mol/L disodium ethylenediaminetetraacetate VS, and add 50mL of water, 3mL of ammonia-ammonium chloride burr solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride reagent. Titrate the solution with the prepared zinc acetate solution, until the blue color changes to blue-purple. Calculate the molarity factor.

Zinc Acetate, 0.02 mol/L
1000 mL of this solution contains 4.390 g of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$: 219.51].
Preparation Dissolve 4.43 g of zinc acetate dihydrate in 20mL of water and 2mL of dilute acetic acid, add water to make 1000mL, and standardize the solution as follows:
Standardization Proceed as directed for standardization under 0.05 mol/L zinc acetate VS, but measure exactly 20 mL of 0.02 mol/L disodium ethylenediaminetetraacetate VS.

Zinc Sulfate, 0.1 mol/L
1000mL of this solution contains 28.756 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7HyO$: 287.56).
Preparation Dissolve 28.8 g of zinc sulfate for volumetric analysis in water to make 1000mL, and standardize the solution as follows:
Standardization Pipet 25mL of the prepared zinc sulfate solution, add 5mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate with 0.1 mol/L disodium ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue-purple. Calculate the molarity factor.

(4) Standard Solutions

Borate pH Standard Solution See the pH Determination.

Calcium Hydroxide pH Standard Solution See the pH Determination.

Carbonate pH Standard Solution See the pH Determination.

Oxalate pH Standard Solution See the pH Determination.

Phosphate pH Standard Solution See the pH Determination.

pH Standard Solution, Borate See the pH Determination.

pH Standard Solution, Calcium Hydroxide See the pH Determination.

pH Standard Solution, Carbonate See the pH Determination.

pH Standard Solution, Oxalate See the pH Determination.

pH Standard Solution, Phosphate See the pH Determination.

pH Standard Solution, Phthalate See the pH Determination.

Phthalate pH Standard Solution See the pH Determination.

Standard Aluminum Stock Solution Weigh 1.0 g of aluminum, add 60mL of diluted hydrochloric acid (1 in 2), dissolve by heating, cool, add water to make 1000mL. Pipet 10mL of this solution, add 30mL of water and 5mL of acetic acid-ammonium acetate burr solution, pH 3.0, and adjust the pH to about 3 with ammonia TS added dropwise. Then, add 0.5mL of Cu-PAN TS, and titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS under boiling until the

color of the solution changes from red to yellow lasting for more than 1 minute. Perform a brank determination.

Each mL of 0.01 mol/L disodium ethylenediaminetetraacetate VS = 0.26982 mg of Al

Standard Ammonium Solution Dissolve 2.97 g of ammonium chloride, exactly weighed, in purified water for ammonium limit test to make exactly 1000mL. Measure exactly 10mL of this solution, and add purified water for ammonium limit test to make exactly 1000mL: 1mL of this solution contains 0.01 mg of ammonium (NH_4).

Standard Arsenic Solution See the Arsenic Limit Test.

Standard Arsenic Stock Solution See the Arsenic Limit Test.

Standard Boron Solution Weigh exactly 0.286 g of boric acid, previously dried in a desiccator (silica gel) to constant mass, and dissolve in water to make exactly 1000mL. Pipet 10mL of this solution, and add water to make exactly 1000mL: 1mL of this solution contains 0.5 μg of boron (B).

Standard Cadmium Solution Measure exactly 10mL of Standard Cadmium Stock Solution, and add diluted nitric acid (1 in 3) to make exactly 1000mL. Pipet 10mL of this solution, and add diluted nitric acid (1 in 3) to make 100mL: 1mL of this solution contains 0.001 mg of cadmium (Cd). Prepare before use.

Standard Cadmium Stock Solution Dissolve 1.000 g of cadmium ground metal, exactly weighed, in 100mL of dilute nitric acid by gentle heating, cool, and add dilute nitric acid to make exactly 1000mL.

Standard Calcium Solution Weigh exactly 0.250 g of calcium carbonate, add 5mL of dilute hydrochloric acid and 25mL of water, and dissolve by heating. After cooling, add water to make exactly 1000mL: 1mL of this solution contains 0.1 mg of calcium (Ca).

Standard Copper Solution Pipet 10mL of Standard Copper Stock Solution, dilute with water to make exactly 1000mL: 1mL of this solution contains 0.01 mg of copper (Cu). Prepare before use.

Standard Copper Stock Solution Weigh exactly 1.000g of copper (standard reagent), add 100mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000mL.

Standard Cyanide Solution Measure exactly a volume of Standard Cyanide Stock Solution, equivalent to 10 mg of cyanide (CN), add 100mL of sodium hydroxide TS and water to make exactly 1000mL: 1mL of this solution contains 0.01 mg of cyanide (CN). Prepare before use.

Standard Cyanide Stock Solution Dissolve 2.5 g of potassium cyanide in water to make exactly 1000mL. Measure exactly 100mL of this solution, add 0.5mL of 4-dimethylaminobenzylidenerhodanine TS, and titrate with 0.1 mol/ L silver nitrate VS until the solution shows a red color.

Each mL of 0.1 mol/L silver nitrate VS = 5.204 mg of CN

Standard Fluorine Solution See the Oxygen Flask Combustion Method.

Standard Gold Solution for Atomic Absorption Spectrophotometry To 25mL of Standard Gold Stock Solution, exactly measured, add water to make exactly 1000mL: 1mL of this solution contains 0.025 mg of gold (Au).

Standard Gold Stock Solution Dissolve 0.209 g of chlorauric acid, exactly weighed, in 2mL of aqua regia, heat on a water bath for 10 minutes, and add 1 mol/L hydrochloric acid TS to make exactly 100mL: 1mL of this solution contains 1.00 mg of gold (Au).

Standard Iron Solution Weigh exactly 86.3 mg of ammonium iron (III) sulfate 12-hydrate, dissolve in 100mL of water, and add 5mL of dilute hydrochloric acid and water to make exactly 1000mL: 1mL of this solution contains 0.01 mg of iron (Fe).

Standard Lead Solution Measure exactly 10mL of Standard Lead Stock Solution, and add water to make exactly 100mL: 1mL of this solution contains 0.01 mg of lead (Pb). Prepare before use.

Standard Lead Stock Solution Weigh exactly 159.8 mg of lead (II) nitrate, dissolve in 10mL of dilute nitric acid, and add water to make exactly 1000mL. Prepare and store this solution using glass containers, free from soluble lead salts.

Standard Mercury Solution Weigh exactly 0.0135 g of mercury (II) chloride, previously dried for 6 hours in a desiccator (silica gel), dissolve in 10mL of dilute nitric acid, and add water to make exactly 1000mL. Pipet 10mL of this solution, and add 10mL of dilute nitric acid and water to make exactly 1000mL: 1mL of this solution contains 0.1 g of mercury (Hg). Prepare before use.

Standard Methanol Solution See the Methanol Test.

Standard Nickel Solution Dissolve 6.73 g of ammonium nickel (II) sulfate hexahydrate, exactly weighed, in water to make exactly 1000mL. Pipet 5mL

of this solution, add water to make exactly 1000mL: 1mL of this solution contains 0.005 mg of nickel (Ni).

Standard Nitric Acid Solution Weigh exactly 0.0722 g of potassium nitrate, dissolve in water to make exactly 1000mL: 1mL of this solution contains 0.01 mg of nitrogen (N).

Standard Phosphoric Acid Solution Weigh exactly 0.358 g of monobasic potassium phosphate, previously dried to constant mass in a desiccator (silica gel), and add 10mL of diluted sulfuric acid (3 in 10) and water to make exactly 1000mL. Pipet 10mL of this solution, and add water to make exactly 100mL: 1mL of this solution contains 0.025 mg of phosphoric acid (as PO_4).

Standard Phosphate Solution

Standard Potassium Stock Solution Weigh exactly 9.534 g of potassium chloride, previously dried at 130 °C for 2 hours, and dissolve in water to make exactly 1000mL: 1mL of this solution contains 5.00 mg of potassium (K).

Standard Silver Solution for Atomic Absorption Spectrophotometry Measure exactly 10mL of Standard Silver Stock Solution, and add water to make exactly 1000mL: 1mL of this solution contains 0.01 mg of silver (Ag). Prepare before use.

Standard Silver Stock Solution Dissolve 1.575 g of silver nitrate, exactly weighed, in water to make exactly 1000mL: 1mL of this solution contains 1.00 mg of silver (Ag).

Standard Sodium Dodecylbenzene Sulfonate Solution Weigh exactly 1.000 g of sodium dodecylbenzene sulfonate, and dissolve in water to make exactly 1000mL. Pipet 10mL of this solution, and add water to make exactly 1000mL: 1mL of this solution contains 0.01mg of sodium dodecylbenzene sulfonate [$CH_3(CH_2)_{11}C_6H_4SO_3Na$].

Standard Sodium Stock Solution Weigh exactly 2.542 g of sodium chloride (standard reagent), previously dried at 130 °C for 2 hours, and dissolve in water to make exactly 1000mL: 1mL of this solution contains 1.00 mg of sodium (Na).

Standard Tin Solution Weigh exactly 0.250 g of tin, and dissolve in 10mL of sulfuric acid by heating. After cooling, transfer this solution with 400mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500mL. Pipet 10mL of this solution, and add diluted hydrochloric acid (1 in 5) to make exactly 1000mL: 1mL of this solution contains 0.005 mg of tin (Sn). Prepare before use.

Standard Tyrosine Solution See the monograph Pancreatin.

Standard Vinyl Chloride Solution Transfer about 190mL of ethanol for gas chromatography into a 200mL volumetric flask, and stopper with a silicone rubber stopper. Cooling this volumetric flask in a methanol-dry ice bath, inject 0.20 g of vinyl chloride, previously dried, through the silicone rubber stopper, and then inject ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, through the silicone rubber stopper to make 200mL. Then pipet 1mL of this solution, add ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, to make exactly 200mL. Pipet 1mL of this solution, add ethanol for gas chromatography, cooled previously in a methanol-dry ice bath to make exactly 100mL. Preserve in a hermetic container, at a temperature not exceeding -20 °C.

Standard Water-Methanol Solution See the Water Determination.

Standard Zinc Solution Measure exactly 25mL of Standard Zinc Stock Solution, and add water to make exactly 1000mL. Prepare before use. OnemL of this solution contains 0.025 mg of zinc (Zn).

Standard Zinc Solution for Atomic Absorption Spectrophotometry See the Test for Rubber Closure for Aqueous Infusions

Standard Zinc Stock Solution Dissolve 1.000 g of zinc (standard reagent), exactly weighed, in 100mL of water and 5mL of hydrochloric acid with the aid of gentle heating, cool, and add water to make 1000mL.

(5) Matching Fluids for Color

Matching Fluids for Color are prepared from the following colorimetric stock solutions. Colorimetric stock solutions are prepared by the following procedures and stored in glass-stoppered bottles. When the color of the solution is compared with Matching Fluids for Color, unless other wise specified, transfer both solutions and fluids to Nessler tubes and view transversely against a white background.

Cobaltous Chloride Colorimetric Stock Solution Weigh 65 g of cobalt (II) chloride hexahydrate, and dissolve in 25mL of hydrochloric acid and water to make 1000mL. Measure exactly 10mL of this solution, and add water to make exactly 250mL. Measure exactly 25mL of the solution, add 75mL of water and 0.05 g of mulexide-sodium chloride indicator, and add dropwise diluted ammonia solution (28) (1 in 10) until the color of the solution changes from red-purple to yellow. Titrate with 0.01 mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes, after the addition of diluted ammonia solution

(28) (1 in 10) near the endpoint, from yellow to red-purple.

Each mL of 0.01 mol/L disodiumethylenediaminetetraacetate VS = 2.3793 mg of $CoCl_2 \cdot 6H_2O$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make 1mL contains 59.5 mg of cobalt (II) chloride hexahydrate (CoCl2·6H2O: 237.93), and use this solution as the colorimetric stock solution.

Cupric Sulfate Colorimetric Stock Solution Weigh 65 g of copper (II) sulfate pentahydrate, and dissolve in 25mL of hydrochloric acid and water to make 1000mL. Measure exactly 10mL of this solution, and add water to make exactly 250mL. Measure exactly 25mL of this solution, add 75mL of water, 10mL of a solution of ammonium chloride (3 in 50), 2mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of mulexide-sodium chloride indicator. Titrate with 0.01mol/L disodium ethylenediaminetetraacetate VS until the color of the solution changes from green to purple.

Each mL of 0.01 mol/L disodiumethylenediaminetetraacetate VS = 2.4969 mg of $CuSO_4 \cdot 5H_2O$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make 1mL contains 62.4 mg of copper (II) sulfate pentahydrate (CuSO4·5H2O: 249.69),and use this solution as the colorimetric stock solution.

Matching fluid for color	Parts of cobaltous chloride colorimetric stock solution (mL)	Parts of ferric chloride colorimetric stock solution (mL)	Parts of Cupric Sulfate calorimetric stock solution (mL)	Parts of water (mL)
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0..4	1.2	0.3	3.1
F	0.3	1.2	-	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	-	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	-	-
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	-	4.9	0.1	-
O	0.1	4.8	0.1	-
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	-	4.7
T	0.5	0.5	0.4	3.6

Ferric Chloride Colorimetric Stock Solution Weigh 55 g of iron (III) chloride hexahydrate, and dissolve in 25mL of hydrochloric acid and water to make 1000mL. Measure exactly 10mL of this solution, transfer to an iodine flask, add 15mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100mL of water to the mixture, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS = 27.030 mg of $FeCl_3 \cdot 6H_2O$

According to the titrated value, add diluted hydrochloric acid (1 in 4) to make 1mL contain 45.0 mg of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$: 270.30), and use this solution as the colorimetric stock solution.

Matching Fluids for Color Measure exactly the volume of colorimetric stock solutions and water as shown in the above table with a buret or a pipet graduated to less than 0.1mL, and mix.

(6) Optical Filters for Wavelength and Transmission Rate Calibration

CRM 204-06-100
KRISS(Korea Research Institute of Standards and Science) CRM 204-06-100 Optical filters for transmission rate calibration
Transmission rates: 10 %, 50 %, 90 %
Wavelength range: 270 nm ~ 700 nm
Uncertainty: ±1 %

CRM 204-08-100
KRISS(Korea Research Institute of Standards and Science) CRM 204-08-100 Optical filters for wavelength calibration
Validation: Record absorption peaks with given wavelength widths in the the following wavelength range, using a standard spectrophotometer.
Wavelength range: 380 nm ~ 780 nm, 13 steps
Uncertainty: ±2 nm (wavelength width: 2.0 nm), ±0.5 nm (wavelength width: 5.0 nm)

(7) Measuring Instruments, Appliances

Thermometers Ordinarily, use calibrated thermometers with an immersion line (rod) or calibrated total immersion mercury-filled hermometers according to the Korean Industrial Standards. Use the thermometers with the immersion line (rod), shown in the following table, for the tests in Congealing Point, Melting Point (Method 1), Boiling Point and Distilling Range.

Volumetric measures Use volumetric flasks, pipets, burets and measuring cylinders conforming to the Korean Industrial Standard.

Cassia flask Use glass-stoppered flasks, shown in Fig. 1, made of hard glass and having graduation lines of volume on the neck.

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen	Nitrogen
Temperature range	- 17 - 50 °C	40 - 100 °C	90 - 150 °C	140 - 200 °C	190 - 250 °C	240 - 320 °C
Minimum graduation	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C
Longer graduation lines at	each 1 °C	each 1 °C	each 1 °C	each 1 °C	each 1 °C	each 1 °C
Graduation numbered at	each 2 °C	each 2 °C	each 2 °C	each 2 °C	each 2 °C	each 2 °C
Total length (mm)	280 -300	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300
Stem diameter (mm)	6.0 ±0.1	6.0 ±0.1	6.0 ±0.1	6.0 ±0.1	6.0 ±0.1	6.0 ±0.1
Bulb length (mm)	12 – 15	12 - 15	12 - 15	12 - 15	12 - 15	12 – 15
Distance from bottom of bulb to graduation at the lowest temperature (mm)	75 – 90	75 - 90	75 - 90	75 - 90	75 - 90	75 – 90
Distance from top of thermometer to graduation at the highest temperature (mm)	35 – 50	35 - 50	35 - 50	35 - 50	35 - 50	35 – 50
Distance from bottom of bulb to immersion line (mm)	60	60	60	60	60	60
Form of top of thermo-meter	loop	loop	loop	loop	loop	loop
Maximum scale error at any point	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.2 °C	0.4 °C

Nessler tube Use colorless, glass-stoppered cylinders 1.0 to 1.5 mm in thickness, shown in Fig. 2, made of hard glass. The difference of the height of the graduation line of 50mL from the bottom among cylinders does not exceed 2 mm.

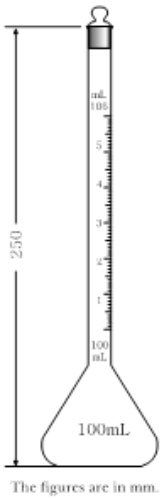


Fig. 1

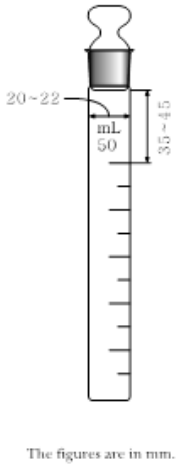


Fig. 2

Balances and masses **(1)** ***Chemical balances*** Use balances readable to the extent of 0.1 mg.
(2) ***Semimicrobalances*** Use balances readable to the extent of 0.01 mg.
(3) ***Microbalances*** Use balances readable to the extent of 0.001 mg.
(4) ***Masses*** Use calibrated masses.

Gas mixer Use the apparatus, showm in Fig. 3, made of hard glass.

A: Gas buret (capacity of 100mL, about 13.7 mm in inside diameter, graduated in 0.2mL divisions, and graduated in 0.1mL divisions at the lower, narrow part)

B: Gas buret (capacity of 100mL, about 4.2 mm in inside diameter at the upper stem with graduation in 0.02mL divisions, about 28.5 mm in inside diameter at the lower stem with graduation in 1mL divisions)

C: (C_1,C_2,C_3 and C_4): Three-way stopcock

D: Inlet of sample (bent forward at 20 mm in length)

E: Outlet of mixed gas (bent forward at 20 mm in length)

F: Jacket (about 770 mm in length, about 40 mm in outside diameter, almost completely filled with water at room temperature)

G: Rubber pressure tubing, about 4 mm in inside diameter (G_1: about 80 cm in length; G_2 and G_3. about 120 cm in length)

H: Heavy-wall capillary tube (about 1 mm in inside diameter)

K: Receiver

L: Leveling bulb (L_1. filled with about 50mL of mercury; L_2 and L_3. filled with about 150mL of mercury)

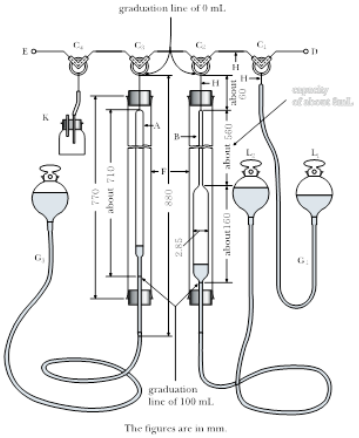


Fig. 3 Gas Mixer

Sieves Sieves conform to the specifications in the following table. Use the sieve number of nominal size as the designation.

Sieve Number	nominal size (μm)	Specification of sieves					
		Sieve opening (mm)			Wire (mm)		
		Size (mm)	Permissible variation		dia-meter	Permissible variation	
			Average	Maximum		Maximum	Minimum
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 Manipulation, and Ultrafiltration

(1) Sterilization and Aseptic Manipulation

Sterilization

Sterilization means a process whereby the destruction or removal of all living microorganisms is accomplished. Usually sterilization is accomplished by one or a combination of the following methods, generally depending on the kind of microorganism, the conditions of contamination and the quality and nature of the substance to be sterilized. In addition the sterilization process requires the choice of appropriate procedure and accurately controlled operation and conditions.

The adequacy of sterilization is decided by means of the Sterility Test.

The procedure for sterilization should be carried out after confirming that the temperature, pressure, etc. are adequate for the desired sterilization.

For the choice of the conditions for sterilization or verification of the integrity of sterilization, biological indicators suitable for individual conditions of sterilization may be used.

Aseptic manipulation

Aseptic manipulation is a technique used for processing sterile pharmaceutical products which re not sterilized in their final containers, and applied to a series of aseptic processes of sterile products which are prepared by the filtration sterilization and/or with sterile raw materials.

The aseptic manipulation requires the presterilization of all of the equipments and materials used for processing sterile products, and then the products are processed in a way to give a defined sterility assurance level in the aseptic processing facilities where microbial and particulate levels are adequately manipulated.

(2) Ultrafiltration

The Ultrafiltration is a water filtration method by means of crucial flow filtration utilizing either a reverse osmotic membrane or an ultrafilter, or an apparatus combining both.

When the Water for Injection is prepared by the Ultrafiltration, pretreatment facilities, facilities for preparation of water for injection, and facilities for supplying water for injection are usually used. The pretreatment facilities, placed before the preparation facilities, are used to remove solid particles, dissolved salts and colloids in original water, so as to reduce load on the preparation facilities. They are assemblies having a cohesion apparatus, precipitation-separation apparatus, filtration apparatus, chlorine sterilization apparatus, oxidation-reduction apparatus, residual chlorine removing apparatus, precise filtration apparatus, reverse osmosis apparatus, ultrafiltration apparatus, ion exchange apparatus, etc., which are combined properly depending upon the quality of original water. The facilities for preparing water for injection consist of a pretreatment water supplying apparatus, ultraviolet sterilization apparatus, heat exchange apparatus, membrane module, cleansing-sterilization apparatus, etc. The facilities for supplying water for injection consist of a reservoir with a capacity to meet changing demand tubes for distributing Water for Injection, heat exchange apparatus, circulation pump, pressure control apparatus, etc. Usually, the Water for Injection prepared by the Ultrafiltration circulates in the facilities at a temperature not lower than 80 °C for prevention of microbial proliferation.

For preparing the Water for Injection by means of the Ultrafiltration, use a membrane module which removes microorganisms and substances of molecular masses not less than approximately 6000.