

# GENERAL TESTS, PROCESSES AND APPARATUS

General Tests, Processes and Apparatus includes common methods for tests, useful test methods for quality recognition of drugs and other articles related to them. Unless otherwise specified, acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, amino acid analysis of proteins, ammonium determination, arsenic determination, atomic absorption spectrophotometry, boiling point determination, chloride determination, conductivity measurement, congealing point determination, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, distilling range determination, endpoint determination in titrimetry, flame coloration, fluorometry, foreign insoluble matter test for injections, foreign insoluble matter test for ophthalmic solutions, gas chromatography, heavy metal determination, infrared spectrophotometry, insoluble particulate matter test for injections, insoluble particulate matter test for ophthalmic solutions, iron determination, liquid chromatography, loss on drying determination, loss on ignition determination, melting point determination, methanol determination, microbial assay for antibiotics, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolarity determination, oxygen flask combustion method, particle size determination, particle size distribution test for preparations, pH determination, powder particle density determination, qualitative test, refractive index determination, residual solvents test, residue on ignition determination, specific gravity and density determination, specific surface area determination, sulfate determination, test for bacterial endotoxins, test for glass containers for injections, test for metal particles in ophthalmic ointments, test for microbial limit, test for microbial limit for crude drugs, test for plastic containers, test for pyrogen, test for readily carbonizable substances, test for rubber closure for aqueous infusions, test for sterility, test for total organic carbon, test of extractable volume for injection, thermal analysis, thin-layer chromatography, ultraviolet-visible spectrophotometry, uniformity of dosage units (test for content uniformity, mass variation test), viscosity determination, vitamin A assay, water determination, and X-ray powder diffraction are performed as directed in the corresponding articles under the General Tests, Processes and Apparatus. The tests for melting point of fats, congealing point of fatty acids, specific gravity, acid value, saponification value, ester value, hydroxyl value, unsaponifiable matter and iodine value of fats and fatty oils are performed as directed in the corresponding items under Fats and Fatty Oils Test, and sampling, preparation of sample for analysis, microscopic examination, purity test, loss on drying, total ash, acid-insoluble ash, extract content, and essential oil content of crude drugs are performed as directed in the corresponding items under Crude Drugs Test.

The number of each test method is a category number given individually. The number in brackets (< >) appeared

in monograph indicates the number corresponding to the general test method.

## 1. Chemical Methods

### 1.01 Alcohol Number Determination

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

#### 1. 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 10 mL of a sample measured at 15°C by the following procedures.

##### 1.1. Apparatus

Use hard glass apparatus as illustrated in Fig. 1.01-1. Ground glass may be used for the joints.

##### 1.2. Reagent

Alkaline phenolphthalein solution: To 1 g of phenolphthalein add 7 mL of sodium hydroxide TS and water to make 100 mL.

##### 1.3. Procedure

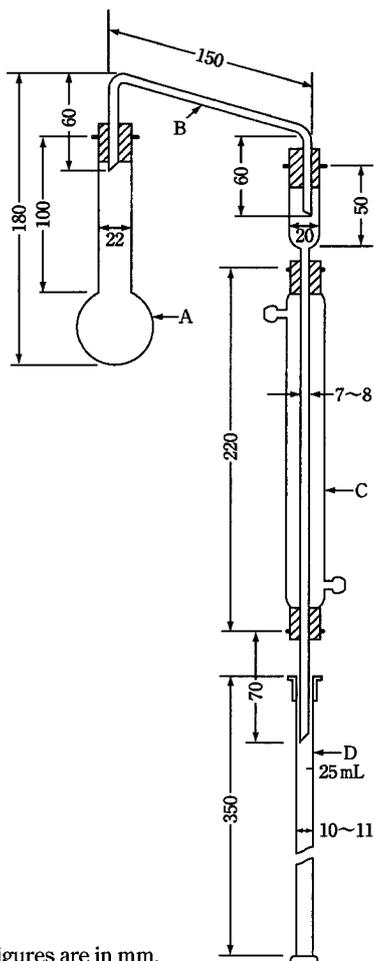
Transfer 10 mL of the sample preparation, accurately measured at  $15 \pm 2^\circ\text{C}$ , to the distilling flask A, add 5 mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder D.

By reference to Table 1.01-1, 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.

Table 1.01-1

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



The figures are in mm.

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

Fig. 1.01-1

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, diethyl ether or camphor require treatment as follows. Mix 10 mL of the sample, accurately measured, with 10 mL of saturated sodium chloride solution in a separator, add 10 mL of petroleum benzin, and shake. Collect the separated aqueous layer. The petroleum benzin layer was extracted with two 5 mL portions 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 3 mL more than that shown in the above 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  $15 \pm 2^\circ\text{C}$  for 30 minutes, read the volume of the upper reddish ethanol layer in mL, 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.

## 2. Method 2 Gas chromatography

This is a method to determine the alcohol number by determining ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) content (vol%) from a sample measured at  $15^\circ\text{C}$  by the following procedures.

### 2.1. Reagent

Ethanol for alcohol number: Ethanol (99.5) with determined ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) content. The relation between specific gravity  $d_{15}^{15}$  of ethanol and content of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) is 0.797:99.46 vol%, 0.796:99.66 vol%, and 0.795:99.86 vol%.

### 2.2. Preparation of sample solution and standard solution

Sample solution: Measure accurately a volume of sample at  $15 \pm 2^\circ\text{C}$  equivalent to about 5 mL of ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ), and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

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

### 2.3. Procedure

Place 25 mL each of the sample solution and the standard solution in a 100-mL, narrow-mouthed, 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 1 mL each of the gas in the bottle with a syringe according to the Gas Chromatography <2.02> 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.

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

$$\times \frac{\text{ethanol (C}_2\text{H}_5\text{OH) content (vol\%) of ethanol for alcohol number}}{9.406}$$

*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- to 180- $\mu\text{m}$  porous ethylvinylbenzene-divinylbenzene copolymer (mean pore

size: 0.0075  $\mu\text{m}$ , 500 – 600  $\text{m}^2/\text{g}$ ) 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 1 mL 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 the resolution between these peaks being not less than 2.0.

## 1.02 Ammonium Limit Test

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

In each monograph, the permissible limit for ammonium (as  $\text{NH}_4^+$ ) is described in terms of percentage (%) in parentheses.

### 1. Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Fig. 1.02-1. For the distillation under reduced pressure, use the apparatus shown in Fig. 1.02-2. Either apparatus are 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.

### 2. Procedure

#### 2.1. Preparation of test solution and control solution

Unless otherwise specified, test solutions and control solu-

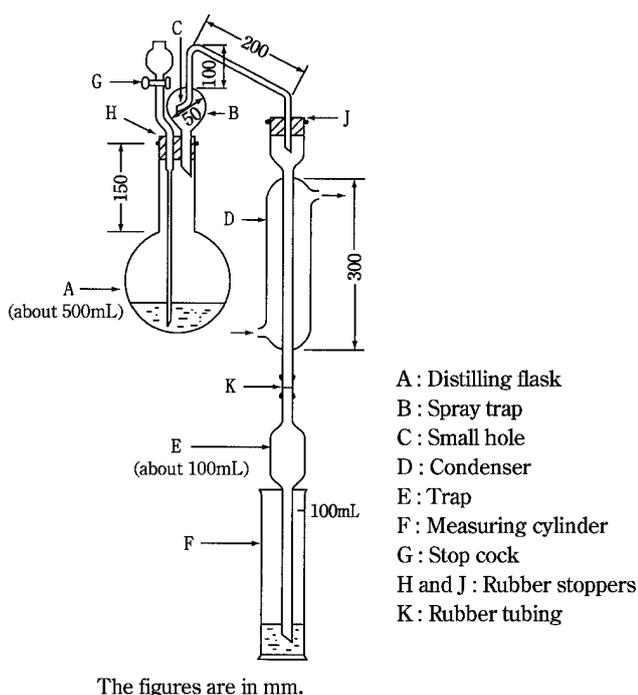


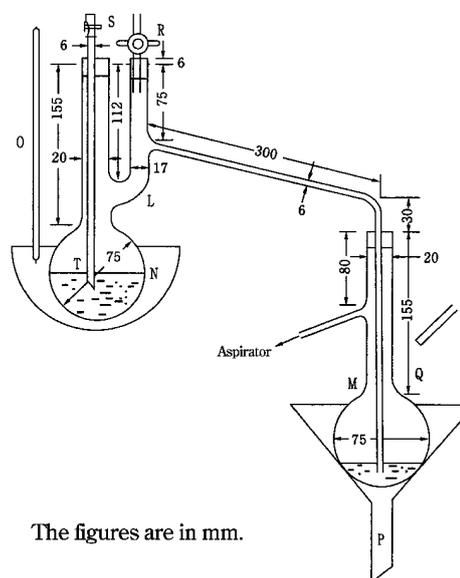
Fig. 1.02-1 Distilling apparatus for ammonium limit test

tion are prepared as directed in the following.

Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver (measuring cylinder) F add 20 mL 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 7 mL per minute of distillate, and distill until the distillate measures 60 mL. 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 100 mL and designate it as the test solution.

For the distillation under reduced pressure, take the amount of sample specified in the monograph to the vacuum distillation flask L, add 70 mL of water and 1 g of magnesium oxide, and connect to the apparatus (Fig. 1.02-2). To the receiver M add 20 mL of a solution of boric acid (1 in 200) as 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 2 mL per minute, and continue the distillation until to get 30 mL 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 100 mL, and perform the test using this solution as the test solution.

Place a volume of Standard Ammonium Solution, directed in the monograph, in the distilling flask A or the vacuum distillation flask L, proceed as for the preparation



- L: Vacuum distillation flask (200-mL)  
 M: Receiver (a 200-mL flask)  
 N: Water bath  
 O: Thermometer  
 P: Funnel  
 Q: Cooling water  
 R: Glass cock  
 S: Rubber tube with screw cock  
 T: Glass tube for anti-bumping

Fig. 1.02-2 Vacuum distilling apparatus for ammonium limit test

of the test solution, and designate it as the control solution.

### 2.2. Test of the test solution and the control solution

Unless otherwise specified, proceed as directed in the following.

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

## 1.03 Chloride Limit Test

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.

### 1. 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 40 mL. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions by using the same procedure.

Add 1 mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from light. 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.

## 1.04 Flame Coloration Test

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.

(1) **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 sample, make the sample 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 sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

(2) **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 sample 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.

## 1.05 Mineral Oil Test

Mineral Oil Test is a method to test mineral oil in nonaqueous solvents for injections and for eye drops.

### 1. Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, 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.

## 1.06 Oxygen Flask Combustion Method

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.

### 1. Apparatus

Use the apparatus shown in Fig. 1.06-1.

### 2. Preparation of test solution and blank solution

Unless otherwise specified, prepare them by the following method.

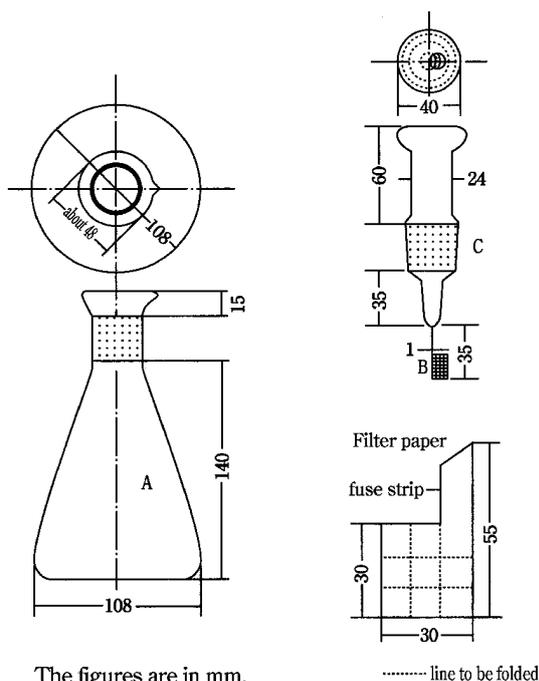
#### 2.1. Preparation of sample

(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.

#### 2.2. 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 figures are in mm.

..... line to be folded

- A: Colorless, thick-walled (about 2 mm), 500-mL 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 gauge. (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.

Fig. 1.06-1

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.

### 3. Procedure of determination

Unless otherwise specified in the monograph, perform the test as follows.

#### 3.1. 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 dropwise until a yellow color develops, then add 25 mL of 2-propanol, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS  
= 0.1773 mg of Cl

Each mL of 0.005 mol/L silver nitrate VS  
= 0.3995 mg of Br

#### 3.2. 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 25 mL 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 dropwise until a yellow color develops, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the potentiometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS  
= 0.6345 mg of I

#### 3.3. 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  $30 \mu\text{g}$  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 mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a blank prepared 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.

Amount (mg) of fluorine (F) in the test solution  
= amount (mg) of fluorine in 5 mL of

$$\text{the standard solution} \times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$$

Standard Fluorine Solution: Dry sodium fluoride (standard reagent) in a platinum crucible between  $500^\circ\text{C}$  and  $550^\circ\text{C}$  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.

#### 3.4. 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 <2.50> with 0.005 mol/L sulfuric acid VS. Perform the test with the blank solution in the same manner.

Each mL of 0.005 mol/L barium perchlorate VS  
= 0.1604 mg of S

## 1.07 Heavy Metals Limit Test

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 so-

dium 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.

### 1. Preparation of test solutions and control solutions

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

#### 1.1. Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it 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 2 mL of dilute acetic acid and water to make 50 mL.

#### 1.2. 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 2 mL 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 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL 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 50 mL.

#### 1.3. 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 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL 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 50 mL.

#### 1.4. Method 4

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol

(95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL 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 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500°C and 600°C. Cool, and add 3 mL 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 50 mL.

### 2. 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.

## 1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)

Nitrogen Determination is a method to determine nitrogen 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.

### 1. Apparatus

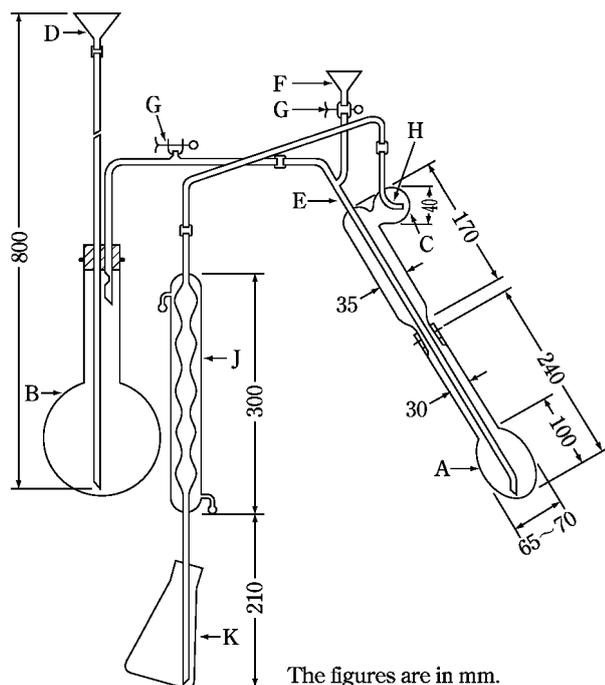
Use the apparatus illustrated in Fig. 1.08-1. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly 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.

### 2. 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 desiccator (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



- A: Kjeldahl flask  
 B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling chips for preventing bumping have been added  
 C: Spray trap  
 D: Water supply funnel  
 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  
 J: Condenser, the lower end of which is beveled  
 K: Absorption flask

Fig. 1.08-1

digestion flask. When the test is performed as directed in the instrumental manual the nitrogen content (%) in amidosulfuric acid should be determined between 14.2% and 14.6%.

### 3. Reagents, Test Solutions

Decomposition accelerator: Unless otherwise specified, use 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of copper (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.

### 4. Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N: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 (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that

the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus (Fig. 1.08-1) washed beforehand by passing stream through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, lose the clamp attached to the rubber tubing G, then begin the distillation with stream, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate <2.50> 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.

$$\begin{aligned} \text{Each mL of 0.005 mol/L sulfuric acid VS} \\ = 0.1401 \text{ mg of N} \end{aligned}$$

If an automated apparatus is used, proceed as directed in the instrumental procedure.

## 1.09 Qualitative Tests

Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5 mL of the test solution.

### Acetate

(1) When warmed with diluted sulfuric 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 (95), 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 red 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 1 mL of ammonium amidosulfate TS, allowed to stand for 1 minute, and then mixed with 1 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS, exhibit 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 added 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 by addition of dilute hydrochloric acid.

**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) <1.04> is applied to barium salts, a persistent yellow-green color develops.

(2) Solutions of barium salts produce with dilute sulfuric acid a white precipitate, which does not dissolve upon addi-

tion of dilute nitric acid.

(3) Acidic solutions of barium salts in acetic acid produce a yellow precipitate with potassium chromate TS. The precipitate dissolves by addition of dilute nitric acid.

**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 <2.60>.

(2) Neutral solutions of benzoates produce a pale yellow-red precipitate upon dropwise 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 with potassium iodide TS a black precipitate, which dissolves in an excess of the reagent to give an orange-colored solution.

**Borate**

(1) When ignite a mixture of a borate with sulfuric acid and methanol, it burns 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 dropwise.

**Bromate**

(1) Acidic solutions of bromates in nitric acid yield with 2 to 3 drops of silver nitrate TS a white, crystalline precipitate, which 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 1 mL 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) <1.04> 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.

(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 with magnesium sulfate TS a white precipitate, which dissolves by addition of dilute acetic acid.

(3) Cold solutions of carbonates exhibit a red color with 1 drop of phenolphthalein TS (discrimination from bicarbonates).

#### Ceric 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 by addition of ammonia TS.

(2) When indigocarmine TS is added dropwise 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 dropwise addition of sodium hydrogensulfite 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 (II) acetate TS. When acetic acid (31) 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 dropwise 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 1 mL 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 (II) acetate TS. When acetic acid (31) is added to one 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 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 with potassium hexacyanoferrate (II) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferric salts yield with sodium hydroxide TS a gelatinous, red-brown precipitate, which 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 5-sulfosalicylic acid TS.

#### **Ferricyanide**

(1) Solutions of ferricyanides exhibit a yellow color.

(2) Solutions of ferricyanides yield with iron (II) sulfate TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

#### **Ferrocyanide**

(1) Solutions of ferrocyanides yield with iron (III) chloride TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrocyanides yield with copper (II) sulfate TS a red-brown precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

#### **Ferrous salt**

(1) Slightly acidic solutions of ferrous salts yield with potassium hexacyanoferrate (III) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrous salts yield with sodium hydroxide TS a greenish gray, gelatinous precipitate, which 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 dropwise addition of a solution of 1,10-phenanthroline monohydrate in ethanol (95) (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 1.5 mL 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 hexaammonium heptamolybdate TS in the 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 with sodium hydroxide TS a white precipitate, which 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 with potassium chromate TS a yellow precipitate, which does not dissolve in ammonia TS but dissolves in sodium hydroxide TS subsequently added.

#### **Lithium salt**

(1) When the Flame Coloration Test (1) <1.04> is applied to lithium salts, a persistent red color develops.

(2) Solutions of lithium salts yield with disodium hydrogenphosphate TS a white precipitate, which 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 hydrogenphosphate TS.

(2) Solutions of magnesium salts yield with sodium hydroxide TS a white, gelatinous precipitate. 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 bismuth sodium trioxide.

#### 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 with a small quantity of sodium sulfide TS a black precipitate, which 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 dropwise to neutral solutions of mercuric salts, a red precipitate is produced. The precipitate dissolves in an excess of the reagent.

(4) Acidic solutions of mercuric salts in hydrochloric acid yield with a small quantity of tin (II) chloride TS a white precipitate, which 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 with potassium iodide TS a yellow precipitate, which 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 hydrochloric 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 iron (II) 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 crystals.

(2) Solutions of nitrites, when 2 to 3 drops of potassium iodide TS and dilute sulfuric acid are added dropwise, exhibit a yellow-brown color, and then yield a black-purple precipitate. When the mixture is shaken with 2 mL 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 dropwise, exhibit a dark red color. When the mixture is shaken with 2 mL of diethyl ether, the diethyl ether layer exhibits a red color.

#### Oxalate

(1) When potassium permanganate TS is added dropwise 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 dropwise added potassium permanganate TS, and effervesce to evolve a gas.

#### Phosphate (Orthophosphate)

(1) Neutral solutions of phosphates yield with silver nitrate TS a yellow precipitate, which dissolves upon addition of dilute nitric acid or ammonia TS.

(2) Acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with hexaammonium heptamolybdate 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 with magnesia TS a white, crystalline precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

#### Potassium salt

(1) When the Flame Coloration Test (1) <1.04> 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 hydrogen tartrate 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 (31)

yield a yellow precipitate with sodium hexanitrocobaltate (III) 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 <2.60> 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 dropwise.

#### 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 with potassium chromate TS a red precipitate, which dissolves upon addition of dilute nitric acid.

(3) Solutions of silver salts yield a brownish gray precipitate with ammonia TS added dropwise. When ammonia TS is added dropwise 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) <1.04> 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 nonluminous 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 dropwise 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 dropwise 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 nonluminous 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 dropwise 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 dropwise 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 with barium chloride TS a white precipitate, which does not dissolve upon addition of dilute nitric acid.

(2) Neutral solutions of sulfates yield with lead (II) acetate TS a white precipitate, which 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

(1) Most kinds of sulfides evolve the odor of hydrogen sulfide with dilute hydrochloric acid. This gas blackens lead (II) acetate paper moistened with water.

#### Sulfite and Bisulfite

(1) When iodine TS is added dropwise to acidic solutions of sulfites or bisulfites in acetic acid (31), 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 a solution of resorcinol (1 in 50) and 2 to 3 drops of a solution of potassium bromide (1 in 10) with 5 mL 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 3 mL 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 ammonia solution (28) is added to another portion, the precipitate dissolves.

(2) Solutions of thiocyanates produce with iron (III) chloride TS a red color, which is not decolorized by addition of hydrochloric acid.

#### Thiosulfate

(1) When iodine TS is added dropwise to acidic solutions of thiosulfates in acetic acid (31), 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 with an excess of silver nitrate TS a white precipitate, which 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 hexacyanoferrate (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 1 mL of potassium thiocyanate TS are added.

## 1.10 Iron Limit Test

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 permissible limit for iron (as Fe) is described in terms of ppm in parentheses.

### 1. Preparation of test solutions and control solutions

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

#### 1.1. Method 1

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

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

#### 1.2. Method 2

Weigh the amount of sample specified in individual monograph, add 10 mL 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 dropwise until the solution develops a pale red color. Add 20 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

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

#### 1.3. 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 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. After dissolving by warming, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate 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 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL 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.

### 2. Procedure

Unless otherwise specified, proceed as follows:

#### 2.1. Method A

Transfer the test solution and the control solution to separate Nessler tubes, to each add 2 mL of a solution of L-ascorbic acid (1 in 100), mix well, and allow to stand for 30 minutes. Add 1 mL of a solution of  $\alpha$ ,  $\alpha'$ -dipyridyl in ethanol (95) (1 in 200), add water to make 50 mL, 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.

#### 2.2. Method B

Dissolve 0.2 g of L-ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1 mL of a solution of  $\alpha$ ,  $\alpha'$ -dipyridyl in ethanol (95) (1 in 200), and allow to stand for 30 minutes. Then add 2 mL of a solution of 2,4,6-trinitrophenol (3 in 1000) and 20 mL 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.

## 1.11 Arsenic Limit Test

Arsenic Limit Test is a limit test for arsenic contained in drugs. The limit is expressed in terms of arsenic (III) trioxide ( $As_2O_3$ ).

In each monograph, the permissible limit for arsenic (as  $As_2O_3$ ) is described in terms of ppm in parentheses.

### 1. Apparatus

Use the apparatus illustrated in Fig. 1.11-1.

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 (II) 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 J to hold the tube C vertically. Make the lower end to the exit tube of C level with that of the rubber stopper J.

### 2. Preparation of the test solution

Unless otherwise specified, proceed as directed in the following.

#### 2.1. Method 1

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

#### 2.2. Method 2

Weigh the amount of the sample directed in the monograph, add 5 mL of water, and add 1 mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10 mL of sulfurous acid solution, 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 2 mL in volume. Dilute with water to make 5 mL, and designate it as the test solution.

#### 2.3. Method 3

Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 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 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

#### 2.4. Method 4

Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 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 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

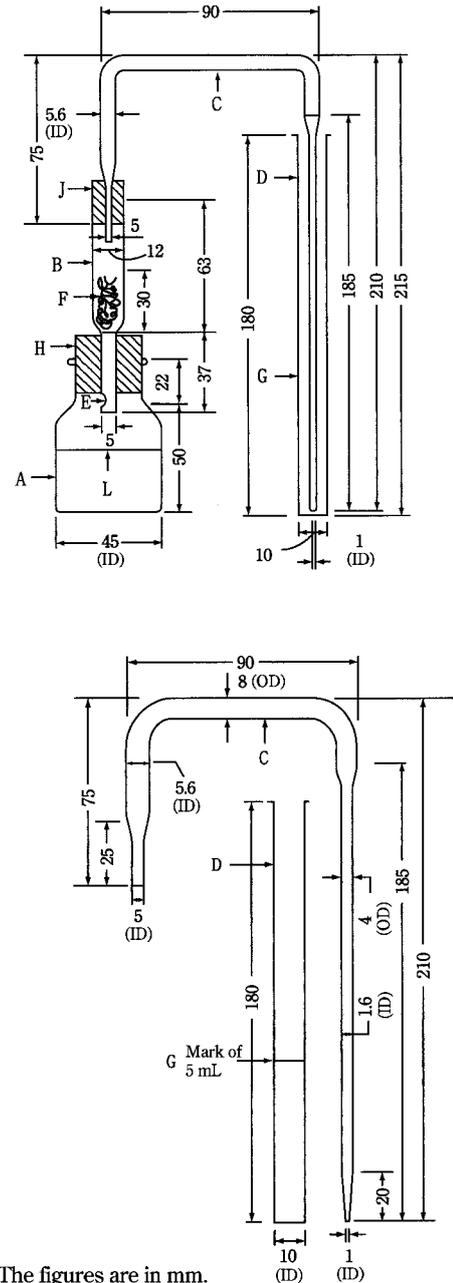
#### 2.5. Method 5

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

### 3. Test solutions

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

Standard Arsenic Stock Solution: Weigh accurately 0.100 g of finely powdered arsenic (III) trioxide dried at 105°C for 4 hours, and add 5 mL of sodium hydroxide solu-



The figures are in mm.

- A: Generator bottle (capacity up to the shoulder: approximately 70 mL)
- B: Exit tube
- C: Glass tube (inside diameter: 5.6 mm, 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 5 mL
- H and J: Rubber stoppers
- L: Mark of 40 mL

Fig. 1.11-1 Arsenic limit test apparatus

tion (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL.

Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1  $\mu\text{g}$  of arsenic (III) trioxide ( $\text{As}_2\text{O}_3$ ). Prepare Standard Arsenic Solution just before use and preserve in a glass-stoppered bottle.

#### 4. Procedure

Unless otherwise specified, proceed using apparatus shown in Fig. 1.11-1. 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 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, and allow to stand for 10 minutes. Then add water to make 40 mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 mL 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 5 mL, 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 2 mL of Standard Arsenic Solution in the generator bottle A. Add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The color produced corresponds to 2  $\mu\text{g}$  of arsenic (III) trioxide ( $\text{As}_2\text{O}_3$ ) and is used as the standard.

#### 5. Note

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

## 1.12 Methanol Test

Methanol Test is a method to determine methanol adhering in ethanol.

#### 1. Reagents

(i) Standard Methanol Solution—To 1.0 g of methanol, accurately measured, add water to make exactly 1000 mL. To 5 mL of this solution, exactly measured, add 2.5 mL of methanol-free ethanol and water to make exactly 50 mL.

(ii) Solution A—To 75 mL of phosphoric acid add water to make 500 mL, then dissolve 15 g of potassium permanganate in this solution.

(iii) Solution B—Add sulfuric acid carefully to an equal volume of water, cool, and dissolve 25 g of oxalic acid dihydrate in 500 mL of this dilute sulfuric acid.

#### 2. Procedure

Pipet 1 mL of the sample, and add water to make exactly 20 mL. Use this solution as the sample solution. Transfer 5 mL each of the sample solution and the Standard Methanol Solution, accurately measured, to test tubes, add 2 mL of Solution A to each solution, and allow to stand for 15 minutes. Decolorize these solutions by adding 2 mL of Solution B, and mix with 5 mL of fuchsin-sulfurous acid TS. Allow to stand for 30 minutes at ordinary temperature. The sample solution has no more color than the Standard Methanol Solution.

## 1.13 Fats and Fatty Oils Test

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

#### 1. 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.

#### 2. Melting point

Proceed by the method described in Method 2 of Melting Point Determination <2.60>.

#### 3. Congealing point of fatty acids

##### 3.1. Preparation of fatty acids

Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1-L 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 500 mL of hot water, and add slowly 50 mL 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 layer 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.

##### 3.2. Measurement of congealing point

Proceed by the method described in Congealing Point Determination <2.42>.

#### 4. Specific gravity

##### 4.1. Liquid sample at ordinary temperature

Proceed by the method described in Determination of Specific Gravity and Density <2.56>.

##### 4.2. 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 Determination of Specific Gravity and Density <2.56>.

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

*M*: Mass (g) of the empty pycnometer

*M*<sub>1</sub>: Mass (g) of the pycnometer filled with the sample

*M*<sub>2</sub>: Mass (g) of the pycnometer filled with water

*M*<sub>3</sub>: Mass (g) of the pycnometer filled with the sample and water

## 5. 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.

### 5.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100 mL 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 <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds. If the sample 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.

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

## 6. 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.

### 6.1. Procedure

Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25 mL 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 1 mL of phenolphthalein TS, and titrate <2.50> immediately the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS. If the sample solution is turbid at lower temperature, titration should be done while warm. Perform a blank determination.

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

Table 1.13-1

Acid value	Amount (g) of sample
Less than 5	20
5 to 15	10
15 to 30	5
30 to 100	2.5
More than 100	1.0

*a*: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed in the blank determination

*b*: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample

## 7. Ester value

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

### 7.1. Procedure

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

## 8. 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.

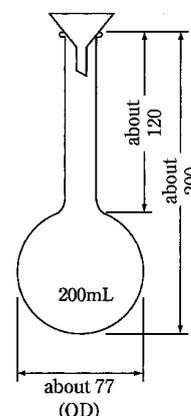
### 8.1. Procedure

Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in Fig. 1.13-1), and add exactly 5 mL 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 1 mL 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 5 mL of neutralized ethanol down into the flask, and titrate <2.50> with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL 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 (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed in the blank determination

*b*: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed for titration of the sample



The figures are in mm.

Fig. 1.13-1 Hydroxyl value determination flask

### 9. Unsaponifiable matter

Unsaponifiable matter is calculated as the difference between the amount of materials, which are unsaponifiable by the procedure described below, soluble in diethyl 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.

#### 9.1. Procedure

Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100 mL of warm water, and transfer the washing to the separator. Further, add 50 mL of water to the separator, and cool to room temperature. Wash the flask with 100 mL 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 50 mL 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 50 mL 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 30 mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolphthalein 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 3 mL 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 2 mL of diethyl ether and 10 mL of neutralized ethanol, and dissolve the residue by shaking well. Add a few drops of phenolphthalein TS, and titrate <2.50> 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.

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

*a*: Amount (g) of the extracts

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

### 10. 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.

#### 10.1. Procedure

Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-2, according to the expected iodine value of the sample, in a small glass container. In a

Table 1.13-2

Iodine value	Amount (g) of sample
Less than 30	1.0
30 to 50	0.6
50 to 100	0.3
More than 100	0.2

500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL 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 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and shake. Then, titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

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

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

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

## 1.14 Sulfate Limit Test

Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO<sub>4</sub>) is described in terms of percentage (%) in parentheses.

### 1. Procedure

Unless otherwise specified, transfer the quantity of the sample, 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 the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure.

Add 2 mL of barium chloride TS to the test solution and to 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 of the control solution.

## 1.15 Readily Carbonizable Substances Test

Readily Carbonizable Substances Test is a method to examine the minute impurities contained in drugs, which are readily colored by addition of sulfuric acid.

**1. 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.

**2. Physical Methods****Chromatography****2.01 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 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.

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

The ratio  $k$  represents the mass distribution ratio 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 ( $t_R$ : 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.

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

**1. 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 ther-

mostat 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 spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few  $\mu\text{g}$ . The recorder is used to record the output signals of the detector. As required, a data processor 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 stepwise or gradient fashion.

**2. 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 sample 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.

**3. Identification and purity test**

When Liquid Chromatography is used for identification of a component of a sample, it is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component 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, highly specific identification can be achieved by confirming identity of the chemical structure of the component and that of an authentic specimen, in addition to the identity of their retention times.

When Liquid Chromatography is used for purity test, it is generally performed by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using 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 its relative response factor to the principal component.

#### 4. Assay

##### 4.1. 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 sample 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 sample 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.

##### 4.2. 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 sample 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 sample 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.

#### 5. Method for peak measuring

Generally, the following methods are used.

##### 5.1. 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 both sides of the peak.

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

##### 5.2. 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.

#### 6. 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 tests, the evaluation of "Test for required detectability" may also be required.

##### 6.1. 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 tests, "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.

##### 6.2. 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 of 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.

### 6.3. 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.

### 7. Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material, column temperature, composition ratio of the mobile phase, composition of buffer solutions in the mobile phase, pH of the mobile phase, concentration of ion-pair forming agents in the mobile phase, ionic strength of the mobile phase, flow rate of the mobile phase, number and timing of mobile phase composition changes in gradient program, flow rate of mobile phase in gradient program, composition and flow rate of derivatizing reagents, and reaction time and chamber temperature in chemical reaction may be modified within the ranges in which the liquid chromatographic system used conforms to the requirements of system suitability.

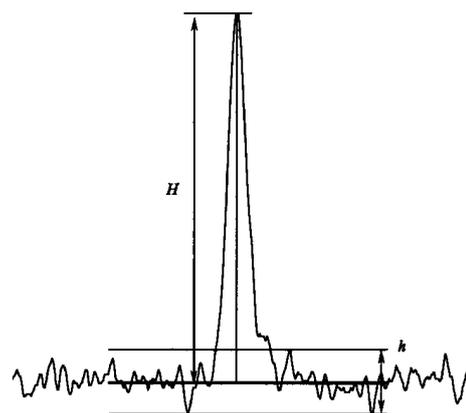
### 8. Terminology

(i) **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 elutes.

(ii) **Symmetry factor:** It shows the degree of symmetry of a peak in the chromatogram, and is defined as *S* in the following equation.

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

*W*<sub>0.05h</sub>: 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*<sub>0.05h</sub> and *f* have the same unit.

(iii) **Relative standard deviation:** Generally, it is defined as *RSD* (%) in the following equation.

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

*x*<sub>*i*</sub>: Observed value

$\bar{X}$ : Mean of observed values

*n*: Number of replicate measurements

(iv) **Complete separation of peak:** It means that the resolution between two peaks is not less than 1.5. It is also called as "baseline separation".

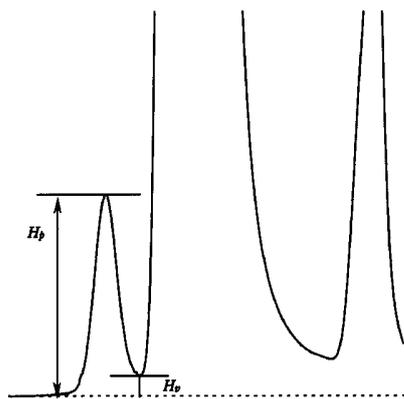
(v) **Peak-valley ratio:** It indicates the degree of separation between 2 peaks on a chromatogram when baseline separation cannot be attained, and is defined as *p/v* by the following formula.

$$p/v = \frac{H_p}{H_v}$$

*H*<sub>*p*</sub>: peak height from the baseline of the minor peak

*H*<sub>*v*</sub>: height from the baseline of the lowest point (peak valley) of the curve between major and minor peaks

(vi) **Separation factor:** It shows the relation between the retention times of peaks in the chromatogram, and is defined as  $\alpha$  in the following equation.



$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

$t_{R1}$ ,  $t_{R2}$ : Retention times of two compounds used for the resolution measurement ( $t_{R1} < t_{R2}$ )

$t_0$ : Time of passage of the mobile phase through the column (time measured from the time of injection of a compound with  $k = 0$  to the time of elution at the peak maximum)

The separation factor ( $\alpha$ ) 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.

(vii) **Resolution:** It shows the relation between the retention time and the peak width of peaks in the chromatogram, and is defined as  $R_S$  in the following equation.

$$R_S = 1.18 \times \frac{t_{R2} - t_{R1}}{W_{0.5h1} + W_{0.5h2}}$$

$t_{R1}$ ,  $t_{R2}$ : Retention times of two compounds used for the measurement of resolution ( $t_{R1} < t_{R2}$ )

$W_{0.5h1}$ ,  $W_{0.5h2}$ : Peak widths at half peak height

Where  $t_{R1}$ ,  $t_{R2}$ ,  $W_{0.5h1}$  and  $W_{0.5h2}$  have the same unit.

(viii) **Number of theoretical plates:** It indicates the extent of band broadening of a compound in the column, and is generally defined as  $N$  in the following equation.

$$N = 5.54 \times \frac{t_R^2}{W_{0.5h}^2}$$

$t_R$ : Retention time of compound

$W_{0.5h}$ : Width of the peak at half peak height

Where  $t_R$  and  $W_{0.5h}$  have the same unit

## 9. Note

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

## 2.02 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 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.

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

### 1. 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 for keeping 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.

### 2. 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 speci-

fied in the individual monograph. Inject a volume of the sample 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.

### 3. Identification and purity test

Identification of a component of a sample is performed by confirming identity of the retention time of the component and that of an authentic specimen, or by confirming that the peak shape of the component is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the peak area of target impurity from the sample solution with that of the main component from a standard solution, which is prepared by diluting the sample solution to a concentration corresponding to the specified limit of the impurity, or by calculating target impurity content using 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 its response factor to the principal component.

### 4. 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.

#### 4.1. 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 sample 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 sample 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.

#### 4.2. 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 sample 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 sample 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.

#### 4.3. Standard addition method

Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area or peak height of individual sample solutions. Calculate the concentration of standard objective compound added into each sample 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.

### 5. Method for peak measuring

Generally, the following methods are used.

#### 5.1. 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.

### 5.2. 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.

### 6. System suitability

Refer to "System suitability" described under 2.01 Liquid Chromatography.

### 7. Point to consider in changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of column, particle size of packing material, concentration or thickness of stationary phase, column temperature, temperature-rising rate, kind and flow rate of carrier gas, and split ratio may be modified within the ranges in which the gas chromatographic system used conforms to the requirements of system suitability. Headspace sample injection device and its operating conditions may be also modified, provided that they give equivalent or more accuracy and precision.

### 8. Terminology

The definition of terms described under 2.01 Liquid Chromatography shall apply in 2.02 Gas Chromatography.

### 9. Note

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

## 2.03 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.

### 1. Preparation of thin-layer plate

Generally, proceed by the following method.

A smooth and uniformly thick glass plate having a size of 50 mm × 200 mm or 200 mm × 200 mm is used for preparing a thin-layer plate. Using a suitable apparatus, apply a water suspension of powdered solid substance for the stationary phase, 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.

### 2. Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample 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.

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:

$$R_f = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

## 2.04 Amino Acid Analysis of Proteins

Amino acid analysis of proteins refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

### 1. Hydrolysis of Protein and Peptide

Acid hydrolysis at 110°C for 24 hours using 6 mol/L hydrochloric acid containing phenol (Method 1) is the most common method for hydrolyzing protein and samples. The result should be analyzed carefully because several amino acids are chemically modified during the acid hydrolysis and thus not recovered quantitatively. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Peptide bonds involving isoleucine and valine are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively.

The hydrolysis techniques, Methods 2 to 11, are used to address these concerns. Some of the hydrolysis techniques, Methods 4 to 11, may cause modifications of cysteine, methionine, asparagines and glutamine to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis by Method 1.

(i) Method 1: Hydrolysis using hydrochloric acid containing phenol (liquid phase hydrolysis, vapor phase hy-

drolisis)

Prevention of tryptophan oxidation

(ii) Method 2: Mercaptoethanesulfonic acid hydrolysis (vapor phase hydrolysis)

(iii) Method 3: Hydrolysis using hydrochloric acid containing thioglycolic acid (vapor phase hydrolysis)

Cysteine-cystine and methionine oxidation

(iv) Method 4: Hydrolysis by Method 1 or Method 2 after oxidation with performic acid

Cysteine-cystine oxidation

(v) Method 5: Hydrolysis using hydrochloric acid containing sodium azide (liquid phase hydrolysis)

(vi) Method 6: Hydrolysis using hydrochloric acid containing dimethylsulfoxide (vapor phase hydrolysis)

Cysteine-cystine reduction and alkylation

(vii) Method 7: Hydrochloric acid hydrolysis after a vapor phase pyridylethylation reaction

(viii) Method 8: Hydrochloric acid hydrolysis after a liquid phase pyridylethylation reaction

(ix) Method 9: Hydrochloric acid hydrolysis after a liquid phase carboxymethylation reaction

Conversion of cysteine-cystine to mixed disulfide

(x) Method 10: Hydrochloric acid hydrolysis after a reaction with dithiodiglycolic acid or dithiodipropionic acid

Derivatization of asparagine and glutamine

(xi) Method 11: Hydrochloric acid hydrolysis after reaction with bis(1,1-trifluoroacetoxy) iodobenzene

A time-course study is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. This technique will allow the analyst to account for some residue destruction.

Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

## 2. Methodologies of Amino Acid Analysis

The amino acid analysis techniques include the postcolumn derivatization for detection (Methods 1 to 2) after the separation of the free amino acids by ion-exchange chromatography and the precolumn derivatization of the free amino acids (Methods 2 to 7) followed by reversed-phase HPLC.

(i) Method 1: Ninhydrin

(ii) Method 2: *o*-Phthalaldehyde (OPA)

(iii) Method 3: Phenylisothiocyanate (PITC)

(iv) Method 4: 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)

(v) Method 5: (Dimethylamino)azobenzenesulfonyl chloride (DABS-Cl)

(vi) Method 6: 9-Fluorenylmethyl chloroformate (FMOC-Cl)

(vii) Method 7: 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F)

Among these methods, ion-exchange chromatography with postcolumn ninhydrin derivatization is one of the most common methods employed for quantitative amino acid analysis. The choice of any one technique often depends on

the sensitivity required from the assay. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary according to the exact equipment and procedure used.

## Spectroscopic Methods

### 2.21 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  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ , etc. These nuclei have intrinsic spin angular momentum, of which the magnitude is given by  $I(I + 1)/h/2\pi$ , where  $I$  is the spin quantum number and is integral or half-integral ( $I = 1/2$  for  $^1\text{H}$  and  $^{13}\text{C}$ ). When the magnetic nuclei are placed in a magnetic field, they are oriented in  $2I + 1$  possible orientations corresponding to  $2I + 1$  equally spaced energy levels (two energy levels for  $^1\text{H}$  and  $^{13}\text{C}$ ). The transition between two successive quantized energy levels corresponding to adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency  $\nu$  is given by

$$\nu = \gamma \cdot \frac{H_0}{2\pi}$$

where  $H_0$  is the strength of the applied external magnetic field and  $\gamma$  is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since the absorption coefficient (the transition probability) does not depend on the environment in which the nuclei are located, the intensity is basically proportional to the number of nuclei. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by a characteristic time constant (known as the relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. 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 ( $\delta$ ), which is independent of the strength of the magnetic field, and is given by

$$\delta = \frac{\nu_S - \nu_R}{\nu_R} + \delta_R$$

where,

$\nu_S$ : The resonance frequency of the observed signal,

$\nu_R$ : The resonance frequency of the reference signal,

$\delta_R$ : The chemical shift of the reference signal (in the case of the value not being 0).

The chemical shifts are normally expressed in ppm, a

dimensionless 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.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei through chemical bonds, resulting in an additional splitting of the signal. The spacing between two adjacent 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 the multiplet pattern more complex.

From the NMR spectrum the following four parameters can be obtained: chemical shift, spin-spin coupling constant, resonance intensity (intensities of  $^1\text{H}$  are proportional to the number of nuclei and those of  $^{13}\text{C}$  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.

## 1. Spectrometer

There are two types of spectrometers.

### 1.1. Fourier transform NMR (FT-NMR) spectrometers (Fig. 2.21-1)

Target nuclei are simultaneously excited in all frequency range of the nuclei by means of an intense radio frequency pulse. The FID (free induction decay) after the pulse is detected, which is a time domain signal, is converted to a frequency domain spectrum by Fourier transformation. Number of data points suitable for the spectral range, flip angle, acquisition time, delay time and number of scans should be set appropriately.

Recently FT-NMR is commonly used because of its high sensitivity and various advanced applications.

### 1.2. Continuous wave NMR (CW-NMR) spectrometers (Fig. 2.21-2)

In the case of the CW method, a spectrum is obtained by sweeping the radio frequency or magnetic field continuously over the frequency range of the nuclei being observed.

## 2. Measurement

Prior to measurements, the sensitivity and resolution of the instrument must be adjusted to the optimum levels using

a standard sample (ethylbenzene, 1,2-dichlorobenzene or acetaldehyde) dissolved in an appropriate NMR solvent.

The sample dissolved in a suitable solvent is transferred into an NMR tube. The reference compound can be added directly to the sample solution (internal reference), or a sealed capillary tube containing the reference compound can be inserted into the NMR tube (external reference). The sample solutions should be completely homogeneous. In particular, solid contaminants should be removed in order to obtain good spectra. Various deuterated NMR solvents are commonly used for NMR measurement and the following points should be considered in selecting an appropriate solvent: (i) The solvent signals do not overlap with the sample signals. (ii) The sample must be soluble in the solvent selected. (iii) The solvent does not react with the sample. Furthermore, it should be noted that chemical shifts can depend upon the solvent employed, sample concentration and deuterium ion concentration, and that viscous solutions usually give rather broad, poorly resolved spectra.

For the reference standards use the reagents for nuclear magnetic resonance spectroscopy. For  $^1\text{H}$  and  $^{13}\text{C}$  spectra, tetramethylsilane (TMS) is usually used as the reference compound for samples dissolved in organic solvents. For samples dissolved in deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium 3-(trimethylsilyl)propionate (TSP) is used. For other nuclei, nitromethane, trichlorofluoromethane and phosphoric acid are used as reference compounds for  $^{15}\text{N}$ ,  $^{19}\text{F}$  and  $^{31}\text{P}$ , respectively. Furthermore, chemical shifts of residual protons in deuterated solvents and  $^{13}\text{C}$  in the solvent instead of a reference compound can be used for  $^1\text{H}$  and  $^{13}\text{C}$  NMR.

## 3. Record of apparatus and measurement conditions

Type of instrument, frequency, solvent, temperature, sample concentration, reference compound, experimental technique, etc. should be recorded to allow appropriate comparison of spectra, because NMR spectra depend on the measurement conditions.

## 4. Identification

The sample solution is prepared and tested by the method directed in each monograph. Usually in the case of  $^1\text{H}$  NMR, the sample is identified by the following method.

### 4.1. Identification by the use of chemical shift, multiplicity and relative intensity

When chemical shifts, multiplicities and relative intensities

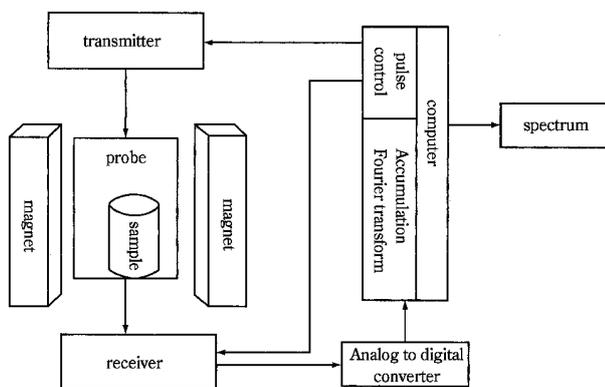


Fig. 2.21-1 FT-NMR spectrometer

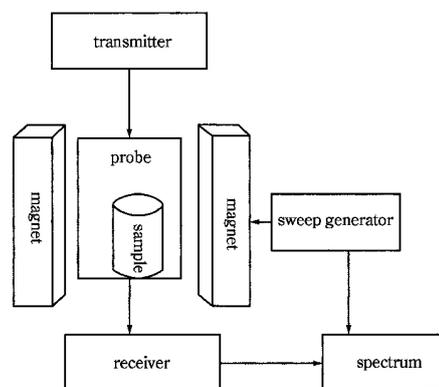


Fig. 2.21-2 CW-NMR spectrometer

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.

#### 4.2. Identification by the use of a Reference Standard

Measurement conditions should be 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 multiplicities and relative intensities of signal at the same chemical shifts, the sample can be identified as being the same substance as the Reference Standard.

### 5. Experimental techniques of $^1\text{H}$ and $^{13}\text{C}$ NMR spectroscopy

NMR spectroscopy includes one-, two- and multi-dimensional techniques, which are used for various purposes.

Spin decoupling, and NOE are available in one-dimensional  $^1\text{H}$  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  $^{13}\text{C}$  spectroscopy. The broadband decoupling technique simplifies a spectrum and achieves enhancement of sensitivity. INEPT (insensitive nuclei enhanced by polarization transfer) and DEPT (distortionless enhancement of polarization transfer) enhance the sensitivity of  $^{13}\text{C}$  by means of polarization transfer from directly bonded  $^1\text{H}$  with a large magnetic moment. They can be applied to identify primary, secondary, tertiary or quaternary 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 two-dimensional 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  $^{13}\text{C}$ - $^{13}\text{C}$  scalar coupling in a sample with natural isotopic abundance, the connectivities of all neighboring  $^{13}\text{C}$  nuclei can be obtained to analyze the carbon skeleton.

HMQC (heteronuclear multiple quantum coherence): This technique observes correlations between  $^1\text{H}$  and  $^{13}\text{C}$  with direct spin-spin coupling using  $^1\text{H}$  detection and reveals intramolecular chemical bonds between hydrogen and carbon atoms.

HMBC (heteronuclear multiple bond connectivity): This technique observes correlations between  $^1\text{H}$  and  $^{13}\text{C}$  with long range spin-spin coupling using  $^1\text{H}$  detection and reveals intramolecular connectivities of hydrogen and carbon atoms.

There are many other techniques such as DQF-COSY (double quantum filtered COSY) and HSQC (heteronuclear single quantum coherence). Furthermore, multidimensional NMR techniques are used to analyze macromolecules.

## 2.22 Fluorometry

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method 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.

$$F = kI_0\phi\epsilon cl$$

$k$ : Constant

$I_0$ : Intensity of exciting light

$\phi$ : Quantum yield of fluorescence or phosphorescence

$$\phi = \frac{\text{number of quanta emitted as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$$

$\epsilon$ : Molar extinction coefficient of the substance at the excitation wavelength

### 1. Apparatus

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

### 2. Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample 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 sample 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, sample, and reference solutions 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 sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

### 3. 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.

## 2.23 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry is a method to determine the amount or the concentration of an element in a sample specimen being examined, by utilizing the phenomenon that atoms being in the ground state absorb the light of specific wavelength, characteristic of the respective atom, when the light passes through an atomic vapor layer of the element to be determined.

### 1. 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 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 non-resonance spectrum, or self-inversion phenomena.

Another special options such as a hydride generator and a heating cell, can also be used for analyzing such as 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.

### 2. Procedure

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

#### 2.1. 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 rate 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 sample so-

lution prepared by the specified procedure is introduced into the flame and the light absorption at the characteristic wavelength of the element to be determined is measured.

#### 2.2. 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. Details of the sample preparation method are provided separately in the monograph.

#### 2.3. 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 sample 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.

### 3. 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.

#### 3.1. 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.

#### 3.2. Standard addition method

To equal volumes of more than 3 sample 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.

### 3.3. 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 sample 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.

### 4. Note

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

## 2.24 Ultraviolet-visible Spectrophotometry

Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under Atomic Absorption Spectrophotometry <2.23>. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity  $I$  to incident light intensity  $I_0$  is called transmittance  $t$ ; transmittance expressed in the percentage is called percent transmission  $T$ , and common logarithm of the reciprocal of transmittance is called absorbance  $A$ .

$$t = \frac{I}{I_0} \quad T = \frac{I}{I_0} \times 100 = 100t \quad A = \log \frac{I_0}{I}$$

The absorbance  $A$  is proportional to the concentration  $c$  of a substance in the solution and the length  $l$  of the layer of the solution through which light passes.

$$A = kcl \quad (k: \text{constant})$$

The constant, calculated on the basis that  $l$  is 1 cm and  $c$  is 1 mol/L, is called molar absorption coefficient  $\epsilon$ . The molar absorption coefficient at the wavelength of maximum absorption is expressed as  $\epsilon_{\max}$ .

When a light beam passes through a substance in the solution, the absorbance by the sample differs depending on the wavelength of the light. So, an absorption spectrum is obtained by determining the absorbances of a light beam at various wavelengths and by graphically plotting the relation

between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption  $\lambda_{\max}$  and that of minimum absorption  $\lambda_{\min}$ .

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determining the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption  $\lambda_{\max}$  and compared it with the absorbance of a standard solution with a certain concentration.

### 1. Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within  $\pm 0.5$  nm. When the measurement is repeated three times, each value obtained should be within the mean  $\pm 0.2$  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 the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within  $\pm 0.3$  nm. When the measurement is repeated three times, each value obtained should be within the mean  $\pm 0.2$  nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean  $\pm 0.002$  when the absorbance is not more than 0.500, and within the mean  $\pm 0.004$  when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

### 2. 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 its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wavelength or over measuring wavelength range after shutting 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, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length 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 the ultraviolet range; use a solvent which does not disturb accurate measurement.

### 3. Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that  $l$  is 1 cm and  $c$  (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as  $E_{1\text{ cm}}^{1\%}$ .

$$E_{1\text{ cm}}^{1\%} = \frac{A}{c \times l}$$

$l$ : Length of the layer of the solution (cm)

$A$ : Absorbance value

$c$ : Concentration of the sample in the solution (w/v%)

The description of, for example, “ $E_{1\text{ cm}}^{1\%}$  (241 nm): 500–530 (after drying, 2 mg, methanol, 200 mL)” in the monograph, indicates that observed  $E_{1\text{ cm}}^{1\%}$  value is between 500 and 530, when the test is performed in the following manner: 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 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

### 4. Identification

Prepare the sample 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 a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

#### 4.1. Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visual Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

#### 4.2. Identification using Reference Standard

When the absorption spectrum obtained from the sample 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 confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

#### 4.3. Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

#### 4.4. Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

### 5. Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample 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.

## 2.25 Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, 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 wave numbers 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 to identify or determine a substance.

### 1. Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2850  $\text{cm}^{-1}$  to the minimum at about 2870  $\text{cm}^{-1}$  should be not less than 18% transmittance and that from the maximum at about 1583  $\text{cm}^{-1}$  to the minimum at about 1589  $\text{cm}^{-1}$  should be not less than 12% transmittance.

The wave number ( $\text{cm}^{-1}$ ) scale is usually calibrated by the

use of several characteristic absorption wave numbers ( $\text{cm}^{-1}$ ) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

3060.0 ( $\pm 1.5$ )	2849.5 ( $\pm 1.5$ )	1942.9 ( $\pm 1.5$ )
1601.2 ( $\pm 1.0$ )	1583.0 ( $\pm 1.0$ )	1154.5 ( $\pm 1.0$ )
1028.3 ( $\pm 1.0$ )		

When the dispersive infrared spectrophotometer is used, the permissible range of the absorption wave numbers at  $1601.2 \text{ cm}^{-1}$  and at  $1028.3 \text{ cm}^{-1}$  should be both within  $\pm 2.0 \text{ cm}^{-1}$ .

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from  $3000$  to  $1000 \text{ cm}^{-1}$ , and the difference of wave number should be within  $5 \text{ cm}^{-1}$  at about  $3000 \text{ cm}^{-1}$  and within  $1 \text{ cm}^{-1}$  at about  $1000 \text{ cm}^{-1}$ .

## 2. 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 of the absorption bands is 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 material is 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  $4000 \text{ cm}^{-1}$  and  $400 \text{ cm}^{-1}$ . The spectrum should be 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.

### 2.1. 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 for 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 in a die with pressure applied to the die of 50 to 100 kN per  $\text{cm}^2$  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.

### 2.2. Solution method

Place the sample 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 sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

### 2.3. Paste method

Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 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.

### 2.4. 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.

### 2.5. Film method

Examine a thin film just as it is or a prepared thin film as directed in each monograph.

### 2.6. 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.

### 2.7. ATR method

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

### 2.8. Diffuse reflectance method

Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about  $50 \mu\text{m}$  particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

## 3. Identification

When the spectrum of a specimen and the Reference Spectrum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

### 3.1. Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, 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.

### 3.2. Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the speci-

men being examined as directed in the monograph, then repeat the measurement.

Infrared Reference Spectra, in the range between  $4000\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$ , are shown at the end of this book for the monographs requiring the identification test by Infrared Spectrophotometry, except for monographs in which "Identification by absorption wave number" is specified.

### 3.3. 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.

## Other Physical Methods

### 2.41 Loss on Drying Test

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% (1 g,  $105^{\circ}\text{C}$ , 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  $105^{\circ}\text{C}$  for 4 hours, and "not more than 0.5% (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.

#### 1. 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  $\pm 10\%$  of the amount directed in the monograph, transfer into the weighing bottle, and, unless otherwise specified, spread the sample 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  $\pm 2^{\circ}\text{C}$  of that directed in the monograph, and, after drying the bottle, the sample 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 sample for 1 to 2 hours to a temperature between  $5^{\circ}\text{C}$  and  $10^{\circ}\text{C}$  below the melting temperature, dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and renew frequently.

## 2.42 Congealing Point Determination

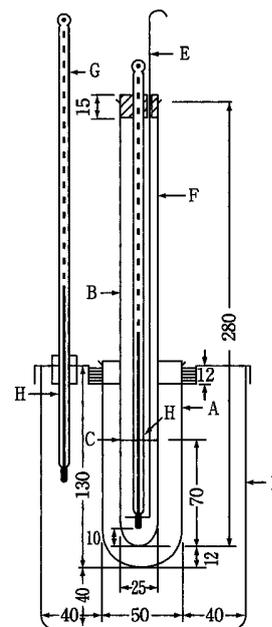
The congealing point is the temperature measured by the following method.

#### 1. Apparatus

Use the apparatus illustrated in Fig. 2.42-1.

#### 2. 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  $20^{\circ}\text{C}$  above the expected congealing point, and transfer to B. Fill the glass or plastic bath D with water at a temperature about  $5^{\circ}\text{C}$  below the expected congealing point. When the sample is liquid at room temperature, fill bath D with water at a temperature between  $10^{\circ}\text{C}$  and  $15^{\circ}\text{C}$  lower than the expected congealing point.



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

Fig. 2.42-1

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.

### 3. 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.

## 2.43 Loss on Ignition Test

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 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.

### 1. Procedure

Previously ignite a crucible or a dish of platinum, quartz or porcelain to 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, reweigh accurately. Use a desiccator (silica gel) for the cooling.

## 2.44 Residue on Ignition Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

♦The Residue on Ignition Test is a method to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

The description, for example, “not more than 0.1% (1 g)”, in a monograph, indicates that the mass of the residue is not more than 1 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accu-

rately and ignited by the procedure described below, and “after drying” indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying. ◆

### 1. Procedure

Ignite a suitable crucible (for example, silica, platinum, quartz or porcelain) at 600 ± 50°C for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately.

Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount (usually 1 mL) 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 residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening 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.

## 2.45 Refractive Index Determination

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 when the first medium is a vacuum 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  $n_D^{20}$ .

### 1. Procedure

For the measurement of refractive index, usually the Abbé

refractometer is used at a temperature in the range of  $\pm 0.2^\circ\text{C}$  of that directed in the monograph. Use of the Abbé 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.

## 2.46 Residual Solvents Test

Residual Solvents Test is a test to determine the amounts of residual organic solvents in pharmaceuticals by using gas chromatography <2.02> or other suitable methods. If only solvents with low toxic potential to man are present in pharmaceuticals, the test of loss on drying <2.41> can be applied in place of gas chromatography.

### 1. Procedure and Test Method

Perform the test by using gas chromatography <2.02> or other suitable methods.

The items necessary for conducting the test should previously be specified so that the test method is suitable for determining the objective residual solvents. For gas chromatography, the items usually necessary to specify are the quantity of sample and reference standard (reference substance) for the test, method for preparing sample and standard solutions, injection amounts of sample and standard solutions to gas chromatograph, calculation formula, operating conditions for head-space apparatus and gas chromatography, and system suitability.

## 2.47 Osmolarity Determination

Osmolarity Determination is a method for measuring the osmotic concentration of the sample solution from the extent of the freezing-point depression.

When a solution and a pure solvent are separated by a semipermeable membrane, through which the solvent can pass freely, but the solute cannot, a part of the solvent passes into the solution compartment through the membrane. The pressure difference produced between the two compartments concomitantly with the solvent migration through the membrane, is defined as the osmotic pressure  $\Pi$  (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, this is not applicable to a solution containing low molecular species, which can pass through a semipermeable membrane. 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 a 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 ionic and neutral species 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 in two ways, one being mass-based concentration (osmolality, mol/kg) and the other, volume-based concentration (osmolarity, mol/L). In practice, the latter is more convenient.

Unless otherwise specified, the freezing-point depression method is used for measuring the osmotic concentration. The method is based on the linear dependency of the freezing-point depression  $\Delta T$  ( $^\circ\text{C}$ ) upon the osmolality  $m$  (mol/kg), as expressed in the following equation,

$$\Delta T = K \cdot m$$

In this equation,  $K$  is the molal freezing-point depression constant, and it is known to be  $1.86^\circ\text{C kg/mol}$  for water. Since the constant  $K$  is defined on the basis of molarity, the molar osmotic concentration can be obtained from the above equation. In the dilute osmotic concentration range, osmolality  $m$  (mol/kg) can be assumed to be numerically equal to osmolarity  $c$  (mol/L). Thus, the conventional osmolarity (mol/L) and the unit of osmole (Osm) are adopted in this test method. One Osm means that the Avogadro number ( $6.022 \times 10^{23}/\text{mol}$ ) of species is contained in 1 L of solution. Usually the osmotic concentration is expressed as the sub-multiple milliosmole (mOsm, mosmol/L) in the Pharmacopoeia.

### 1. Apparatus

Usually, the osmotic concentration of a solution can be obtained by measuring the extent of the freezing-point depression. The apparatus (osmometer) is composed of a sample cell for a fixed volume of sample solution and a cell holder, a cooling unit and bath with a temperature regulator, and a thermistor for detecting temperature.

### 2. Procedure

A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

The apparatus must first be calibrated by the two-point calibration method by using osmolal standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolal standard solutions in the *Table* below, water can also be used as a standard solution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration  $m$  and  $\Delta T$ , the osmolarity of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolarity.

In the case of higher osmolar solutions over 1000 mOsm, dilute the sample by adding distilled water and prepare  $n$  times diluted sample solution (1 in  $n$ ). Measure the osmolarity of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolarity for the sample (see below) is an apparent osmolarity obtained by the dilution method. When the dilution method is applied,

the dilution number should be selected so that the expected osmolarity is nearly equal to that of physiological saline solution.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

### 3. Suitability of the apparatus

After the calibration of the apparatus, a suitability test must be done by repeating the measurement of osmolarity for one of the standard solutions not less than 6 times. In performing the test, it is advisable that the osmolarity of a sample solution and the selected standard solution are similar to each other. In this test, the repeatability of measured values and the deviation of the average from the indicated value should be less than 2.0% and 3.0%, respectively. When the requirement is not met, calibrate the apparatus again by the two-point calibration method, and repeat the test.

### 4. Preparation of the osmolar standard solutions

Weigh exactly an amount indicated in Table 2.47-1 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.

### 5. Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution (NaCl 0.900 g/100 mL)  $c_s$  (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity is  $c_T$  (mOsm), can be calculated by means of the following equation,

$$\text{Osmolar ratio} = \frac{c_T}{c_s}$$

$c_s$ : 286 mOsm

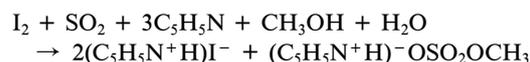
When the measurement is done by the dilution method, because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution  $c_T$  can be calculated as  $n \cdot c'_T = c_T$ , in which  $n$  is the dilution number and  $c'_T$  is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. Thus when the dilution measurement is performed, the dilution number must be stated as (1 in  $n$ ).

Table 2.47-1

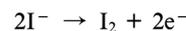
Standard solution for osmometer calibration (milliosmoles)	Amount of sodium chloride (g)
100	0.309
200	0.626
300	0.946
400	1.270
500	1.593
700	2.238
1000	3.223

## 2.48 Water Determination (Karl Fischer Method)

Water Determination 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:



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 Fisher 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.



### 1. Volumetric titration

#### 1.1. 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.

#### 1.2. Reagents

(i) Chloroform for water determination—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 per mL.

(ii) Methanol 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 about 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 per mL.

(iii) 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 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 per mL.

(iv) 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 monoethyl ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

(v) 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 per mL.

(vi) Imidazole for water determination—Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg per g.

(vii) 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 per mL.

### 1.3. Preparation of test solutions and standard solutions

#### 1.3.1. Karl Fischer TS for water determination

The Karl Fischer TS is preserved in a cold place, protecting it from light and moisture.

##### 1.3.1.1. Preparation

Prepare according to the following method (i), (ii) or (iii). Additives may be added for the purpose of improving the stability or 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 100 mL of pyridine for Karl Fischer method, 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 500 mL by adding chloroform for Karl Fischer method or methanol for Karl Fischer method, and allow to stand for more than 24 hours before use.

###### (ii) Preparation 2

Dissolve 102 g of imidazole for Karl Fischer method in 350 mL 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 220 mL of propylene carbonate for water determination until the mass increase of the solvent reaches 32 g. To this solution, add 180 mL of propylene carbonate, or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for Karl Fischer method is dissolved and cooled in ice bath. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

##### 1.3.1.2. Standardization

According to the procedure described below, take a suitable quantity of methanol for Karl Fischer method in a dried titration flask, and titrate the solvent with a Karl Fischer TS to make the inside of the flask anhydrous. Then, weigh about 30 mg of water accurately and put it in the titration flask quickly, and titrate the water dissolved in the solvent with a Karl Fischer TS to the end point, under vigorous stirring. Calculate the water equivalence factor,  $f$  (mg/mL), cor-

responding to the amount of water (H<sub>2</sub>O) in mg per 1 mL of the Karl Fischer TS by using the following equation:

$$f(\text{mg/mL}) = \frac{\text{Amount of water taken (H}_2\text{O) (mg)}}{\text{Volume of Karl Fischer TS consumed for titration of water (H}_2\text{O) (mL)}}$$

##### 1.3.2. Standard water-methanol solution

Standard water-methanol solution is preserved in a cold place, protecting it from light and moisture.

###### 1.3.2.1. 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.

Perform the standardization of this solution, followed by the procedure for Karl Fischer TS. Preserve it in a cool place, protecting it from light and moisture.

###### 1.3.2.2. Standardization

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 10 mL of Karl Fischer TS to this solution in 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:

$$f'(\text{mg/mL}) = \frac{f(\text{mg/mL}) \times 10(\text{mL})}{\text{Volume of the standard water-methanol solution consumed for titration (mL)}}$$

##### 1.4. 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 ( $\mu\text{A}$ ) 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 value 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 two 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 from a polarization state of several hundreds (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 a 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 the 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.

#### 1.4.1. 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 – 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 interfere with the Karl Fisher 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.

$$\text{Water (H}_2\text{O) \%} = \frac{\text{Volume of Karl Fischer TS consumed for titration (mL)} \times f(\text{mg/mL})}{\text{Amount of sample (mg)}} \times 100$$

#### 1.4.2. Back titration

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 – 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 – 30 minutes, with protection from moisture, perform the titration under vigorous stirring.

Water (H<sub>2</sub>O) % =

$$\frac{\left\{ \left[ \begin{array}{l} \text{Volume of Karl} \\ \text{Fischer TS} \\ \text{added (mL)} \end{array} \right] \times f(\text{mg/mL}) - \left[ \begin{array}{l} \text{Volume of the standard water-} \\ \text{methanol solution consumed} \\ \text{for titration (mL)} \end{array} \right] \times f'(\text{mg/mL}) \right\}}{\text{Amount of sample (mg)}} \times 100$$

## 2. Coulometric titration

### 2.1. 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.

### 2.2. Preparation of anolyte and catholyte solutions for water determination

Electrolytic solutions shall consist of an anolyte solution and a catholyte solution, the preparations of which are described below.

#### 2.2.1. Preparation 1

(i) Anolyte for water determination—Dissolve 102 g of imidazole for water determination in 900 mL 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 1000 mL.

(ii) Catholyte for water determination—Dissolve 24 g of diethanolamine hydrochloride in 100 mL of methanol for water determination.

#### 2.2.2. Preparation 2

(i) 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 50 mL 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.

(ii) Catholyte for water determination—Dissolve 30 g of choline hydrochloride into methanol for water determination and adjust the volume to 100 mL by adding the methanol.

#### 2.2.3. Preparation 3

(i) 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 de-

termination (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.

(ii) Catholyte for water determination—Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitroethane (4:1).

### 2.3. 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 – 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 – 5 mg of water to the vessel. After stirring the mixture for 5 – 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 Fisher 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.

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.

$$\text{Water (H}_2\text{O) \%} = \frac{\text{Quantity of electricity required for iodine production (C)}}{10.72 \text{ (C/mg)} \times \text{Amount of sample (mg)}} \times 100$$

10.72: quantity of electricity corresponding to 1 mg of water (C/mg)

## 2.49 Optical Rotation Determination

Optical Rotation Determination is a method for the measurement of the angular rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In case of the ordinary light, the directions of the planes are unrestricted, while in case of the plane polarized light, commonly called as pola-

rized light, the vibrations take place on only one plane that includes the advancing direction of the beam. And it is called that these beams have plane of polarization. Some drugs in the liquid state or in solution have a 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 constitution of the substance.

The optical rotation is a degree of rotation of polarized plane, caused by the optically active substance or its solution, and it is measured by the polarimeter. This value is proportional to the length of the polarimeter tube, and is also related to the solution concentration, the temperature and the measurement wavelength. The character of the rotation is indicated by the direction of the rotation, when facing to the advancing direction of the polarized light. Thus in case of rotation to the right, it is called dextrorotatory and expressed by placing plus sign (+), while in case of rotation to the left, it is called levorotatory and expressed by placing minus sign (–) before the figure of the angular rotation. For example, +20° means 20° of rotation to the right, while –20° means 20° of rotation to the left.

The angular rotation  $\alpha'_x$  means degree of rotation, when it is measured at  $t^\circ\text{C}$  by using specific monochromatic light  $x$  (expressed by wavelength of light source or the specific beam name). 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 lamp.

The specific rotation is expressed by the following equation:

$$[\alpha]_x^t = \frac{\alpha}{lc} \times 100$$

$t$ : The temperature of measurement.

$x$ : The wavelength or the name of the specific monochromatic light (in the case of the Sodium D line, it is described as D).

$\alpha$ : The angle, in degrees, of rotation of the plane of the polarized light.

$l$ : The thickness of the layer of sample solution, *i.e.*, the length of the polarimeter tube (mm).

$c$ : Drug concentration in g/mL. When an intact liquid drug is used for the direct measurement without dilution by an appropriate solvent,  $c$  equals to its density (g/mL). However, unless otherwise specified, the specific gravity is conventionally used in stead of the density.

The description in the monograph, for example, “[ $\alpha$ ]<sub>D</sub><sup>20</sup>: –33.0 – –36.0° (after drying, 1 g, water, 20 mL, 100 mm),” means the measured specific rotation [ $\alpha$ ]<sub>D</sub><sup>20</sup> should be in the range of –33.0° and –36.0°, when 1 g of accurately weighed sample dried under the conditions, specified in the test item of Loss on drying, is taken, and dissolved in water to make exactly 20 mL, then put in the polarimeter tube of 100 mm length, of which temperature is kept at 20°C.

## 2.50 Endpoint 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 a nonaqueous solvent is generally called nonaqueous titration, which is frequently used for volumetric analysis of weak acids, weak bases, and their salts. The endpoint 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 endpoint 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 endpoint 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 endpoint detection methods, there are an electrical potential method and an electrical current method, which are called potentiometric and amperometric titration methods, respectively. They are generically named electrometric titration. In the potentiometric titration method, the endpoint 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 quantity of titrant added. In the amperometric titration method, unless otherwise specified, a bi-amperometric titration method is used, and the endpoint is determined by following the change of microcurrent during the course of a titration. Furthermore, the quantity of electricity (electrical current  $\times$  time) is often used as another electrochemical signal to follow a chemical reaction, as described in Coulometric Titration under Water Determination <2.48>.

The composition of a titration system, such as amount of specimen, solvent, standard solution for volumetric analysis, endpoint detection method, equivalent amount of 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.

### 1. 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 dissolve the specimen. After adding a defined

indicator to the solution to prepare the titrate, titrate by adding a standard solution for volumetric analysis by using a buret. In the vicinity of the endpoint, observe the color change induced by the cautious addition of 0.1 mL or less of the titrant. Calculate the quantity of titrant added from the readings on the scale of the buret used for the titration at the starting point and at the endpoint 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 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.

## 2. Electrical Endpoint Detection Methods

### 2.1. Potentiometric titration

#### 2.1.1. Apparatus

The apparatus consists of a beaker to contain the specimen, a buret for adding a 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 gentle 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 2.50-1 are used according to the kind of titration. As a reference electrode, usually a silver-silver chloride electrode is used. Besides the single indicator electrodes as seen in Table 2.50-1, a combined reference electrode and indicator electrode can also be used.

When the potentiometric titration is carried out by the pH measurement method, the pH meter should be adjusted according to the pH Determination <2.54>.

#### 2.1.2. 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 the buret should be dipped into the solution, to be examined. The endpoint of titration is determined by following the variation of the potential difference between two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of a titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained potential values along the ordinate and the quantity of a titrant added  $V$  (mL) along the abscissa to draw a titration curve, and obtain the endpoint from the

**Table 2.50-1** Kind of Titration and Indicator Electrode

Kind of titration	Indicator electrode
Acid-base titration (Neutralization titration, pH titration)	Glass electrode
Precipitation titration (Titration of halogen ion 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
Nonaqueous titration (Perchloric acid titration, Tetramethylammonium hydroxide titration)	Glass electrode

maximum or the minimum value of  $\Delta E/\Delta V$  or from the value of electromotive force or pH corresponding to the equivalence point.

Unless otherwise specified, the decision of the endpoint 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  $45^\circ$  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 the titration curve. Further, from the intersection point, draw a vertical line to the abscissa, and read the quantity of titrant added as the endpoint of the titration.

Separately, the endpoint 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 endpoint can be determined by following the respective instrumental indications. The endpoint is decided either by following the variation of the differential potential change or the absolute potential difference as a function of the quantity of titrant added: in the former case the quantity given by the maximum or the minimum of the differential values, and in the latter the quantity given by the indicator reaching the endpoint potential previously set for the individual titration system, are assumed to be the endpoint volumes, respectively.

**2.2. Amperometric titration**

**2.2.1. Apparatus**

The apparatus consists of a beaker for holding a specimen, a 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 the two electrodes, 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.

**2.2.2. 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 the buret should be dipped into the solution to be examined. The endpoint of titration is determined by following the changes of microcurrent between the two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of the titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the 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 inflection) as the endpoint 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 sample solution. Determine the amount of the volumetric standard solution needed for giving the endpoint, 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 endpoint in this titration is decided by either of the following methods.

**(i) Drawing method**

Usually, extrapolate the two straight lines before and after the inflection, and obtain the inflection point of the titration curve. Next, read the quantity of titrant added at the inflection point, and assume this point to be the endpoint.

**(ii) Automatic detection method**

In the case of amperometric titration using an automatic titration system, the endpoint can be determined by following the instrumental indications. The endpoint is decided by following the variation of the indicator current during the course of a titration, and the quantity of titrant added is assumed to be that at which the current has reached the endpoint 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.

## 2.51 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  $\kappa$  ( $\text{S}\cdot\text{m}^{-1}$ ) is defined as the reciprocal of resistivity  $\rho$  ( $\Omega\cdot\text{m}$ ), 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 cross-sectional area of a conductor. When resistivity is  $\rho$ , cross-section area  $A$  ( $\text{m}^2$ ), and length  $l$  (m), resistance  $R$  ( $\Omega$ ) can be expressed by the following equation.

$$R = \rho (l/A)$$

Thus, conductivity  $\kappa$  is expressed as follows,

$$\kappa = 1/\rho = (1/R)(l/A)$$

If  $l/A$  is known, the conductivity  $\kappa$  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 ( $\text{S}\cdot\text{m}^{-1}$ ). In practice, conductivity of a solution is generally expressed by  $\mu\text{S}\cdot\text{cm}^{-1}$ , and resistivity by  $\Omega\cdot\text{cm}$ .

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.

### 1. 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  $A$  ( $\text{cm}^2$ ), and the separation distance of the two electrodes is  $l$  (cm), the cell constant  $C$  ( $\text{cm}^{-1}$ ) is given by the following equation.

$$C = \alpha \cdot (l/A)$$

$\alpha$  is 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.

**Table 2.51-1** Conductivity and Resistivity of the Standard Solutions of Potassium Chloride at 20°C

Concentration (g/1000.0 g)	Conductivity $\kappa$ ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Resistivity $\rho$ ( $\Omega\cdot\text{cm}$ )
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

### 2. 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. For the preparation of the standard solutions, take the amount of the dried potassium chloride indicated in Table 2.51-1, dissolve it in distilled water previously boiled and cooled, or water with a conductivity less than  $2\mu\text{S}\cdot\text{cm}^{-1}$ , and adjust to make 1000.0 g. The conductivity and the resistivity of the respective standard solutions at 20°C are shown in Table 2.51-1. These standard solutions should be kept in tightly closed polyethylene or hard glass bottles.

When measurement at 20°C can not be performed, the value of conductivity for the respective standard solution (shown in Table 2.51-1), can be corrected by using the following equation. However, this equation is valid only within the range of 15 – 30°C.

$$\kappa_T = \kappa_{20}[1 + 0.021(T - 20)]$$

$T$ : Measuring temperature specified in the monograph

$\kappa_T$ : Calculated conductivity of the KCl standard solution at  $T^\circ\text{C}$

$\kappa_{20}$ : Conductivity of the KCl standard solution at 20°C

### 3. Operating Procedure

#### 3.1. 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  $0.1\text{cm}^{-1}$ ,  $1\text{cm}^{-1}$ , or  $10\text{cm}^{-1}$ , 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  $20 \pm 0.1^\circ\text{C}$  or at the temperature specified in the monograph, measure the resistance  $R_{\text{KCl}}$  or the conductance  $G_{\text{KCl}}$  of the standard solution, and calculate the cell constant  $C$  ( $\text{cm}^{-1}$ ) by use of the following equation.

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

$R_{\text{KCl}}$ : Measured resistance ( $\text{M}\Omega$ )

$G_{\text{KCl}}$ : Measured conductance ( $\mu\text{S}$ )

$\kappa_{\text{KCl}}$ : Conductivity of the standard solution being used ( $\mu\text{S}\cdot\text{cm}^{-1}$ )

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.

### 3.2. 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  $20 \pm 0.1^\circ\text{C}$ , 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, the relative standard deviation should be less than 2%.

### 3.3. 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  $20 \pm 0.1^\circ\text{C}$  or at the temperature specified in the monograph, measure the resistance  $R_T$  ( $\text{M}\Omega$ ) or conductance  $G_T$  ( $\mu\text{S}$ ) of the sample solution, and calculate the conductivity  $\kappa_T$  by using the following equation.

$$\kappa_T = CG_T \quad \text{or} \quad \kappa_T = C/R_T$$

## 2.52 Thermal Analysis

“Thermal Analysis” is a generic term for a variety of techniques to measure the physical properties of a substance as a function of temperature and/or time.

Among the physical properties, phase transitions such as solid/liquid phase transition (melting, freezing) and crystal polymorphism or thermal behavior such as heat evolution or absorption accompanying thermal degradation or chemical reaction can be detected by the techniques of differential thermal analysis (DTA) or differential scanning calorimetry (DSC). DTA is a method for detecting the thermal behavior of a specimen in terms of the temperature change, while DSC employs the heat quantity (enthalpy) change. There is also a method, thermogravimetry (TG), in which the mass change of a specimen caused by dehydration, adsorption, elimination or oxidation etc., is detected as a function of temperature and/or time.

Among the above three different methods, TG can be used as an alternative method for “Loss on Drying <2.41>” or “Water Determination <2.48>”. However, it must be confirmed beforehand that no volatile component except for water is included in the test specimen when TG is used as an alternative method for “Water Determination”.

### 1. Method 1 Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC)

#### 1.1. Apparatus

Apparatus for DTA or DSC is usually composed of a heating furnace, a temperature-controller, a detector, a device for controlling the atmosphere, and an indicator/recorder.

In a DTA apparatus, a sample specimen and an inert reference material placed in the heating furnace are heated or cooled at a constant rate, and the temperature difference evolved between the sample and reference material is detected continuously by a device such as a thermocouple and recorded as a function of time and/or temperature. As an inert reference material,  $\alpha$ -Alumina for thermal analysis is usually adopted.

Two kinds of DSC apparatus, based upon different principles are available as shown below.

(i) Input compensation-type differential scanning calorimetry (Input compensation DSC): A sample specimen and the 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, which is detected by a device such as a platinum resistance thermometer, is kept at null by controlling the heating unit with a compensation feed-back circuit. The instrument is designed to measure and record continuously the balance of thermal energy applied to each furnace as a function of temperature and/or time.

(ii) Heat flux-type differential scanning calorimetry (Heat flux DSC): A sample specimen and the 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 as a difference of heat flux and recorded as a function of temperature and/or time. In heat flux DSC, thermal conductors are adopted so that the heat flux between the sample and the heat reservoir is proportional to the temperature difference between them.

In usual DSC analysis,  $\alpha$ -Alumina 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.

#### 1.2. Procedure

A sample specimen and the reference material are put in sample pans, and the furnace is heated or cooled under a controlled temperature program. As the temperature changes, the temperature difference (DTA) or heat quantity change (DSC) that develops between the specimen and the reference is detected and recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

A preliminary experiment 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. In this preliminary test, a wide temperature range (room temperature-the temperature at which degradation begins) can be scanned at a rapid heating rate ( $10 - 20^\circ\text{C}/\text{min}$ ). Thereafter, tests by DSC or DTA should be performed at a low heating rate, usually  $2^\circ\text{C}/\text{min}$ , in the chosen temperature range. However, when a clear heat change cannot be observed, such as in a case of glass-transition, the heating rate may be changed to a higher or a lower rate, as

appropriate for the kind of physical change being observed. By analyzing the measured DTA-curve or DSC-curve, a quantity of heat change and/or a specific temperature (ignition, peak or end temperature) that accompanies a physical change, such as melting or polymorphic phase transition, can be obtained.

### 1.3. Calibration of the apparatus

#### 1.3.1. Temperature calibration for DTA and DSC

Temperature calibration for DTA and/or DSC apparatus can be performed by using reference substances having an intrinsic thermal property, such as melting point of pure metals or organic substances, or phase transition point of crystalline inorganic salts or oxides. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

#### 1.3.2. Heat-quantity calibration for DSC

For accurate estimation of a quantity of heat change (enthalpic change) of a sample specimen, caused by a certain physical change accompanying a temperature change, it is necessary to calibrate the apparatus by using appropriate reference substances. As indicated in the section of Temperature calibration, heat-quantity calibration for DSC apparatus can be performed by using appropriate reference substances having a known definite enthalpic change caused by such physical changes as melting of pure metals and/or organic substances, or phase transition of crystalline inorganic salts. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

### 1.4. Notes on operating conditions

When DTA or DSC measurements are made, the following items must be recorded: sample size, discrimination of open- or closed-type sample container, heating or cooling rate, measuring temperature range, and kind and flow rate of atmospheric gas.

## 2. Method 2 Thermogravimetry (TG)

### 2.1. Apparatus

The construction of a TG apparatus is fundamentally similar to that of DTA or DSC apparatus. However, the detector for TG is a balance, called a thermobalance, which can be classified to hanging-, Roberval's-, and horizontal-type balances. The TG apparatus is designed to detect small mass changes of a specimen, placed at a fixed position on a thermobalance, caused by temperature change of the furnace under a controlled temperature program. Mass change with time and/or temperature is recorded continuously.

### 2.2. Procedure

A specimen is put in a sample container, which is placed at a fixed position of the thermobalance, then the heating furnace is run under a controlled temperature program. During this temperature change of the furnace, the mass change of a specimen with time and/or temperature is recorded continuously. Apparatus equipped with a data-processor is operated 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/min, and a linear heating program is recommended. However heating conditions (rate and time span) can be

changed as necessary, depending on the kind of specimen and the extent of the measuring temperature range. Further, in TG measurement, dry air or dry nitrogen is usually passed 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 the TG curve plotted against time and/or temperature, absolute mass change and/or relative mass change with respect to the initial quantity(%) is obtained.

When the mass change caused by oxidation or degradation of a specimen is measured, a specific temperature range has to be determined beforehand so that stable baselines can be obtained before and after a targeted chemical reaction. Subsequent operating procedures are the same as described above.

### 2.3. Calibration of the apparatus

#### 2.3.1. Temperature calibration

The Curie temperature of a ferromagnetic substance such as pure Nickel can be used for temperature calibration for TG, based on the occurrence of an apparent mass change at the Curie point. In the case of a TG apparatus capable of simultaneously conducting DSC and DTA, the same reference substances as those for DTA and DSC can be adopted.

#### 2.3.2. Scale calibration and confirmation

The scale calibration for TG must be done by using reference masses for chemical balances and/or semimicrobalances in the appropriate range. This is called a primary scale calibration, and is performed under ordinary temperature and pressure when the apparatus is set up initially and periodically, thereafter.

In usual measurement by TG, 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 in the real measurement state. This is called secondary scale calibration, and is performed under the standard operation conditions stated below by using the above-mentioned Reference Standard, with a certified water content. 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. However, when the difference is more than 0.3%, scale calibration for TG must be done, based on the certified water content of the Reference Standard.

The standard operation conditions are as follows,

Amount of Calcium Oxalate Monohydrate Reference Standard: 10 mg

Heating rate: 5°C/min

Temperature range: from room temperature to 250°C

Atmospheric gas: dried Nitrogen or dried Air

Flow rate of atmospheric gas,

hanging- or Roberval's-type balance: 40 mL/min

horizontal-type balance: 100 mL/min

### 2.4. Notes on operating conditions

In TG measurement, the following operation conditions must be recorded: sample size, heating rate, temperature range, kind and flow rate of atmospheric gas, etc.

## 2.53 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 a 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,  $\eta$ , is a characteristic of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of Pascal second ( $\text{Pa}\cdot\text{s}$ ), and usually milli-Pascal second ( $\text{mPa}\cdot\text{s}$ ).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity for a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip stress divided by the corresponding slip velocity is called an apparent viscosity. Thus, the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid.

The value of the viscosity,  $\eta$ , divided by the density,  $\rho$ , at the same temperature is defined as a kinematic viscosity,  $\nu$ , which is expressed in the unit of meters squared per second ( $\text{m}^2/\text{s}$ ), and usually millimeters squared per second ( $\text{mm}^2/\text{s}$ ).

The viscosity of a liquid is determined either by the following *Method I* or *Method II*.

### 1. 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 downflowing time of a liquid,  $t$  (s), required for a definite volume of the liquid to flow through a capillary tube is measured and the kinematic viscosity,  $\nu$ , is calculated according to the following equation.

$$\nu = Kt$$

Further, the viscosity,  $\eta$ , is calculated from the next equation:

$$\eta = \nu\rho = Kt\rho$$

where  $\rho$  (g/mL) is the density of the liquid measured at the same temperature,  $t$  ( $^{\circ}\text{C}$ ).

The parameter  $K$  ( $\text{mm}^2/\text{s}^2$ ) represents the viscometer constant and is previously determined by using the *Standard Liquids for Calibrating Viscometers* with known kinematic viscosity. In the case of a liquid having a similar viscosity to water, water itself can be used as a reference standard liquid for the calibration. The kinematic viscosity of water is  $1.0038 \text{ mm}^2/\text{s}$  at  $20^{\circ}\text{C}$ . In the cases of liquids having a slightly higher viscosity than water, the *Standard Liquids for Calibrating Viscometers* should be used for the calibration.

The intrinsic viscosity,  $[\eta]$  (dL/g), of a polymer solution is obtained by plotting the relation of viscosity versus concen-

tration 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 (sample solution) and is also a measure of the average molecular mass of the polymer substance.

The downflowing time  $t$  (s) for a polymer solution, whose concentration is  $c$  (g/dL), and  $t_0$  (s) for the solvent used for dissolving the polymer, 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:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\left(\frac{t}{t_0}\right) - 1}{c} \quad \text{or} \quad [\eta] = \lim_{c \rightarrow 0} \frac{\ln \frac{t}{t_0}}{c}$$

When the concentration dependency of  $\{(t/t_0) - 1\}/c$  is not large, the value of  $\{(t/t_0) - 1\}/c$  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 sample solution is measured with the following apparatus and procedure.

#### 1.1. Apparatus

For measurement of the kinematic viscosity in the range of 1 to  $100,000 \text{ mm}^2/\text{s}$ , the Ubbelohde-type viscometer illustrated in Fig. 2.53-1 can be used. The approximate relations between kinematic viscosity range and inside diameter of the capillary tube suitable for the measurement of various liquids with different viscosity, are given in Table 2.53-1. Although a capillary tube viscometer other than the Ubbelohde-type one specified in Table 2.53-1 can also be used, a viscometer should be selected in which the downflowing time,  $t$  (s), of a sample solution to be determined would be

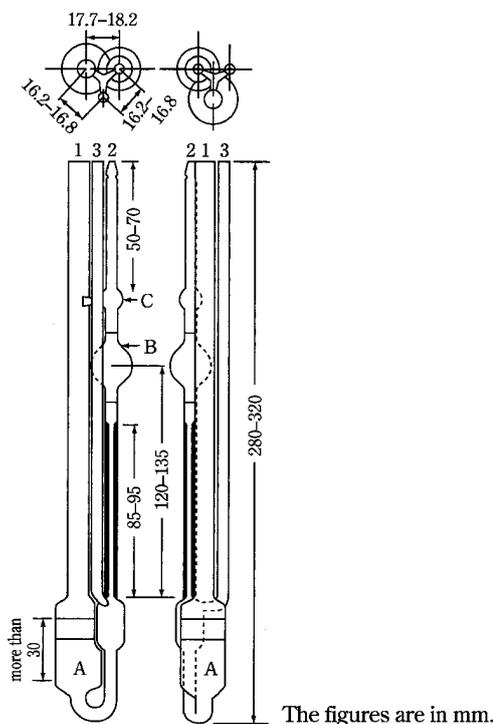


Fig. 2.53-1 Capillary tube viscometer

**Table 2.53-1** Specifications of the Ubbelohde-type viscometer

Viscometer constant $K$ (mm <sup>2</sup> /s <sup>2</sup> )	Inner diameter of capillary tube (mm) Permissible tolerance $\pm 10\%$	Volume of bulb B (mL) Permissible tolerance $\pm 10\%$	Measuring range of kinematic viscosity (mm <sup>2</sup> /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 – 1,000
3.0	2.43	4.0	600 – 3,000
5.0	2.75	4.0	1,000 – 5,000
10.0	3.27	4.0	2,000 – 10,000
30.0	4.32	4.0	6,000 – 30,000
50.0	5.20	5.0	10,000 – 50,000
100	6.25	5.0	20,000 – 100,000

between 200 s and 1000 s.

### 1.2. Procedure

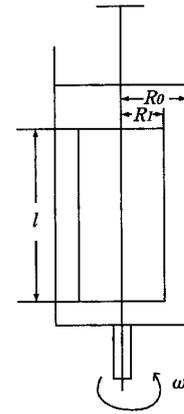
Place a sample solution in a viscometer from the upper end of *tube 1*, so that the meniscus of the solution is at a level between the two marked lines of *bulb A*. Place the viscometer vertically in a thermostatted bath maintained at a specified temperature within 0.1°C, until *bulb C* is fully immersed, and let it stand for about 20 minutes to attain the specified temperature. Close *tube 3* with a finger and pull the sample 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*, and stop the suction. Open the end of *tube 3*, and immediately close the end of *tube 2*. After confirming that the liquid column is cut off at the lowest end of the capillary tube, open the end of *tube 2* to make the sample solution flow down through the capillary tube. Record the time,  $t$  (s), required for the meniscus of the sample solution to fall from the upper to the lower marked line of *bulb B*.

Determine the viscometer constant  $K$  previously, using the *Standard Liquids for Calibrating Viscometers* under the same conditions. The temperature at which the calibration is conducted must be identical with that specified in the monograph.

## 2. 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 extent of torque generated by the rotation can be detected in terms of the torsion of a spring and the liquid viscosity is calculated from the scale-indicated value corresponding to the degree of torsion.

The viscosity of a sample solution is measured with the following apparatus and procedure.

**Fig. 2.53-2a** Coaxial double cylinder-type rotational viscometer

### 2.1. Apparatus

Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

#### 2.1.1. Coaxial double cylinder-type rotational viscometer (Couette type viscometer)

In the coaxial double cylinder-type rotational viscometer, viscosity is determined by placing a liquid in the gap between the inner and the outer cylinders, which share the same central axis and rotate separately, and the generated torque acting on one cylinder surface when the other cylinder is rotated, and the corresponding angular velocity, are measured.

As shown in Fig. 2.53-2a, the inner cylinder is hung by a wire whose twist constant is designated as  $k$ . In Fig. 2.53-2a, 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 gap between the two cylinders, and the outer cylinder is made to rotate at a constant angular velocity,  $\omega$ , the inner cylinder is also forced to rotate due to the viscosity of the liquid. Consequently, torque,  $T$ , is generated by the forced rotation in a viscous liquid, and in the steady state the torque is balanced by the torsion of the wire, as indicated by the degree of rotation  $\theta$ . Then, the relationship can be expressed by  $T = k\theta$  and the viscosity of a liquid,  $\eta$ , is determined from the following equation by measuring the relationship between  $\omega$  and  $\theta$ . Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship holds.

$$\eta = \frac{100T}{4\pi l\omega} \left[ \frac{1}{R_i^2} - \frac{1}{R_o^2} \right]$$

$\eta$ : viscosity of a liquid (mPa·s)

$\pi$ : circumference/diameter ratio

$l$ : length of the inner cylinder (cm)

$\omega$ : 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)

#### 2.1.2. Single cylinder-type rotational viscometer (Brookfield type viscometer)

In the single cylinder-type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder

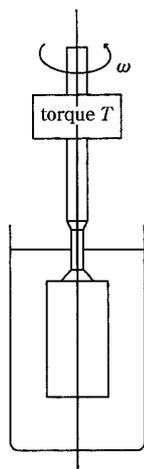


Fig. 2.53-2b Single cylinder-type rotational viscometer

surface when the cylinder immersed in a liquid is rotated at a given angular velocity. Use an apparatus of the type illustrated in Fig. 2.53-2b. If the apparatus constant  $K_B$  is previously determined experimentally by using the *Standard Liquids for Calibrating Viscometers*, the viscosity of a liquid,  $\eta$ , can be obtained from the following equation.

$$\eta = K_B \frac{T}{\omega}$$

where,  $\eta$ : viscosity of a liquid (mPa·s)

$K_B$ : apparatus constant of viscometer (rad/cm<sup>3</sup>)

$\omega$ : angular velocity (rad/s)

$T$ : torque acting on cylinder surface (10<sup>-7</sup> N·m)

### 2.1.3. Cone-flat plate-type rotational viscometer (Cone-plate type viscometer)

In the cone-flat plate-type rotational viscometer, viscosity is determined by placing a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotational axis, and the torque and the corresponding angular velocity are measured, when either the disc or the cone is rotated in a viscous liquid.

As shown in Fig. 2.53-2c, a liquid is introduced to fill the gap between a flat disc and a cone forming an angle  $\alpha$ (rad). When either the flat disc or the cone is rotated at a constant angular velocity or a constant torque, the torque acting on the disc or cone surface rotated by the viscous flow and the corresponding angular velocity in the steady state, are measured. The viscosity of the liquid,  $\eta$ , can be calculated from the following equation.

$$\eta = \frac{3\alpha}{2\pi R^3} \cdot \frac{100T}{\omega}$$

$\eta$ : viscosity of a liquid (mPa·s)

$\pi$ : circumference/diameter ratio

$R$ : radius of cone (cm)

$\alpha$ : angle between flat disc and cone (rad)

$\omega$ : angular velocity (rad/s)

$T$ : torque acting on flat disc or cone surface (10<sup>-7</sup> N·m)

### 2.2. Procedure

Set up the viscometer so that its rotational axis is perpendicular to the horizontal plane. Place a sufficient quantity of

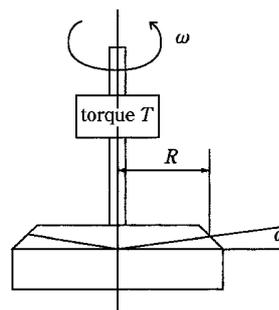


Fig. 2.53-2c Cone-flat plate-type rotational viscometer

a sample solution in the viscometer, and allow the measuring system to stand until a specified temperature is attained, as directed in the monograph. Where it is desired to measure the viscosity within a precision of 1%, measuring temperature should be controlled within 0.1°C. Next, after confirming that the sample solution is at the designated temperature, start operating the rotational viscometer. After the forced rotation induced by the viscous flow has reached a 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  $\eta$  by using the respective equation appropriate to the type of viscometer being used. Determination or confirmation of the apparatus constant should be conducted beforehand by using the *Standard Liquids for Calibrating Viscometers*, and the validation of the apparatus and operating procedure should also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another. From a series of such viscosity measurements, the relationship between the slip velocity and the slip stress 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 or confirmation of the apparatus constant of the rotational viscometer. They are also used for periodic recalibration of the viscometer to confirm maintenance of a specified precision.

## 2.54 pH Determination

pH is defined as the reciprocal of the common logarithm 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 sample solution.

pH of a sample solution is expressed by the following equation in relation to the pH of a standard solution (pH<sub>s</sub>), and can be measured by a pH meter using a glass electrode.

$$\text{pH} = \text{pH}_s + \frac{E - E_s}{2.3026 RT/F}$$

pHs: pH value of a pH standard solution.

$E$ : Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a sample solution:

Glass electrode|sample solution|reference electrode

$E_s$ : Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a pH standard solution:

Glass electrode|standard pH solution|reference electrode

$R$ : Gas constant

$T$ : Absolute temperature

$F$ : Faraday's constant

The value of  $2.3026 RT/F$  (V) in the above equation means the degree of electromotive force (V) per one pH unit and it is dependent on the temperature as shown in Table 2.54-1:

### 1. pH Standard solution

The pH standard solutions are used as a standard of pH, for standardization of a pH meter. To prepare the pH standard solutions, use distilled water or water with a conductivity not more than  $2 \mu\text{S} \cdot \text{cm}^{-1}$  ( $25^\circ\text{C}$ ) and an organic carbon not more than  $0.50 \text{ mg/L}$ , boiled for not less than 15 minutes and cooled in a container fitted with a carbon dioxide-absorbing tube (soda lime). Next, prepare individually 6 kinds of pH standard solutions shown in Table 2.54-2.

Store the pH standard solutions in hard glass or polyethylene bottles. For storage of alkaline pH standard solutions, it is preferable to use a bottle fitted with a carbon dioxide-

absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to ascertain whether the expected pH value is held or not by comparison with newly prepared standard, when the solution is used after long storage.

(i) Oxalate pH standard solution—Pulverize potassium trihydrogen dioxalate dihydrate for pH determination, and dry in a desiccator (silica gel). Weigh  $12.71 \text{ g}$  ( $0.05 \text{ mole}$ ) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(ii) Phthalate pH standard solution—Pulverize potassium hydrogen phthalate for pH determination, and dry at  $110^\circ\text{C}$  to constant mass. Weigh  $10.21 \text{ g}$  ( $0.05 \text{ mole}$ ) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(iii) Phosphate pH standard solution—Pulverize potassium dihydrogen phosphate for pH determination and disodium hydrogen phosphate for pH determination, and dry at  $110^\circ\text{C}$  to constant mass. Weigh  $3.40 \text{ g}$  ( $0.025 \text{ mole}$ ) of potassium dihydrogen phosphate and  $3.55 \text{ g}$  ( $0.025 \text{ mole}$ ) of disodium hydrogen phosphate accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(iv) Borate pH standard solution—Allow sodium tetraborate for pH determination to stand in a desiccator (saturated sodium bromide aqueous solution) until it reaches constant mass. Weigh  $3.81 \text{ g}$  ( $0.01 \text{ mole}$ ) of it accurately, and dissolve in water to make exactly  $1000 \text{ mL}$ .

(v) Carbonate pH standard solution—Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to constant mass, and weigh  $2.10 \text{ g}$  ( $0.025 \text{ mole}$ ) of it accurately. Dry sodium carbonate for pH determination between  $300^\circ\text{C}$  and  $500^\circ\text{C}$  to constant mass, and weigh  $2.65 \text{ g}$  ( $0.025 \text{ mole}$ ) of it accurately. Dissolve both reagents in water to make exactly  $1000 \text{ mL}$ .

(vi) Calcium hydroxide pH standard solution—Reduce calcium hydroxide for pH determination to a fine powder, transfer  $5 \text{ g}$  to a flask, add  $1000 \text{ mL}$  of water, shake well, and allow the solution to become saturated at a temperature between  $23^\circ\text{C}$  and  $27^\circ\text{C}$ . Then filter the supernatant at the same temperature and use the clear filtrate (about  $0.02 \text{ mol/L}$ ).

The pH values of these pH standard solutions at various temperatures are shown in the Table below. pH values at an arbitrary temperature not indicated in Table 2.54-2 can be calculated by the interpolation method.

**Table 2.54-1** Temperature dependency of the electromotive force (V)

Temperature of solution ( $^\circ\text{C}$ )	$2.3026 RT/F$ (V)	Temperature of solution ( $^\circ\text{C}$ )	$2.3026 RT/F$ (V)
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

**Table 2.54-2** pH values of six pH standard solutions

Temperature ( $^\circ\text{C}$ )	Oxalate pH standard solution	Phthalate pH standard solution	Phosphate pH standard solution	Borate pH standard solution	Carbonate pH standard solution	Calcium hydroxide pH standard solution
0	1.67	4.01	6.98	9.46	10.32	13.43
5	1.67	4.01	6.95	9.39	10.25	13.21
10	1.67	4.00	6.92	9.33	10.18	13.00
15	1.67	4.00	6.90	9.27	10.12	12.81
20	1.68	4.00	6.88	9.22	10.07	12.63
25	1.68	4.01	6.86	9.18	10.02	12.45
30	1.69	4.01	6.85	9.14	9.97	12.30
35	1.69	4.02	6.84	9.10	9.93	12.14
40	1.70	4.03	6.84	9.07		11.99
50	1.71	4.06	6.83	9.01		11.70
60	1.73	4.10	6.84	8.96		11.45

### 2. Apparatus

A pH meter generally consists of an electrode system of a glass electrode and a reference electrode, an amplifier and an indicating unit for controlling the apparatus and for displaying the measured value of electromotive force. The indicating unit is usually fitted with dials for zero and span (sensitivity) adjustment. Sometimes a temperature compensation dial is included.

The reproducibility of a pH meter should be within  $0.05 \text{ pH unit}$ , when measurements for an arbitrary pH standard solution are repeated five times, following the procedure described below. After each measurement it is necessary to wash the detecting unit well with water.

### 3. Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement after confirming stable running of the apparatus. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

To standardize 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*. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the sample solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same conditions as used for the first pH standard solution. 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 0.02 pH unit of the tabulated value without further adjustments. When a pH meter is fitted 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 the case of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of two pH standard solutions are identical with the tabulated values within 0.05 pH unit.

After finishing the standardization procedure described above, rinse well the electrodes with water, remove the attached water using a filter paper, immerse the electrode system in the sample solution, and read the indicated pH value after confirming the value is stable. If necessary, a sample solution can be agitated gently.

In the pH determination, the temperature of a sample solution must be controlled to be the same as that of the pH standard solutions with which the pH meter was standardized (within 2°C). When a sample 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 alkali metal ions, an alkali error may be induced in the pH measurement. Thus, in such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

#### 4. Note

Construction and treatment in detail are different for different pH meters.

## 2.55 Vitamin A Assay

Vitamin A Assay is a method to determine vitamin A in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. Method 1 is for the assay of synthetic vitamin A esters, using the ultraviolet-visible spectrophotometry (Method 1-1) or the liquid chromatography (Method 1-2). Method 2 is for the assay of vitamin A of natural origin, containing many geometrical isomers, using the ultraviolet-visible spectrophotometry to determine vitamin A as vitamin A alcohol obtained by saponification in an alkaline solution and extraction.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.300 µg of vitamin A (all-*trans* vitamin A alcohol).

#### 1. Procedure

All procedures should be carried out quickly and care should be taken as far as possible to avoid exposure to light,

air, oxidants, oxidizing catalysts (e.g. copper, iron), acids and heat. If necessary, light-resistant vessels may be used.

Generally, for synthetic vitamin A esters apply Method 1-1 or Method 1-2, but if the assay conditions required for Method 1-1 are not suitable, apply Method 2.

#### 1.1. Method 1-1

Weigh accurately about 0.1 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 50 mL. Dilute this solution with 2-propanol for vitamin A assay to make a solution so that each mL contains 10 to 15 vitamin A Units, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution between 220 nm and 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> to obtain the wavelength of the maximum absorption and the absorbances at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm. When the maximum absorption lies between 325 nm and 328 nm, and the ratios,  $A_{\lambda_i}/A_{326}$ , of each absorbance,  $A_{\lambda_i}$ , at 300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm to the absorbance,  $A_{326}$ , at 326 nm are within the range of  $\pm 0.030$  of the values in the *Table*, the potency of vitamin A in Units per g of the sample is calculated from the following equation.

$$\text{Units of vitamin A in 1 g} = \frac{A_{326}}{M} \times \frac{V}{100} \times 1900$$

$A_{326}$ : Absorbance at 326 nm

$V$ : Total volume (mL) of the sample solution

$M$ : Amount (g) of sample in  $V$  mL of the sample solution

1900: Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

This method is applied to drugs or preparations containing vitamin A esters (retinol acetate or retinol palmitate) as the main component. However, when the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the absorbance ratio  $A_{\lambda_i}/A_{326}$  is not within the range of  $\pm 0.030$  of the values in *Table 2.55-1*, apply Method 2.

#### 1.2. Method 1-2

Proceed with an appropriate amount of sample as directed under Liquid Chromatography <2.01>.

For the assay of retinol acetate and retinol palmitate use Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, respectively, and fix appropriately the operating procedure, the operating conditions and the system suitability based on the characteristics of the substance to be tested and the species and amount of coexisting substances.

#### 1.3. Method 2

Unless otherwise specified, weigh accurately a sample con-

**Table 2.55-1** Absorbance Ratio,  $A_{\lambda_i}/A_{326}$ , of retinol acetate and retinol palmitate

$\lambda_i$ (nm)	$A_{\lambda_i}/A_{326}$	
	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

taining not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL 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 30 mL of water, transfer to a separator A, wash the flask with 10 mL of water and then 40 mL of diethyl ether, transfer the washings to the separator A, shake well, and allow to stand. Transfer the water layer so obtained to a separator B, wash the flask with 30 mL of diethyl ether, add the washing to the separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to the separator A, transfer the water layer in the flask to the separator B, add 30 mL of diethyl ether, and extract by shaking. Transfer the diethyl ether layer so obtained to the separator A, add 10 mL of water, allow the separator A to stand after gentle turning upside-down 2 or 3 times, and remove the water layer. Wash the content of the separator A with three 50-mL portions of water with increasingly vigorous shaking as the washing proceeds. Further wash with 50-mL portions of water until the washing no longer shows a pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, add 5 g of anhydrous sodium sulfate to the flask, mix by shaking, and transfer the diethyl ether to a round-bottomed flask by decantation. Wash the remaining sodium sulfate in the flask with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask. Evaporate the diethyl ether in a water bath at 45°C while swirling the flask, using an aspirator, to about 1 mL, immediately add an exactly measured amount of 2-propanol for vitamin A assay to make a solution containing 6 to 10 vitamin A Units per mL, and designate the solution as the sample solution. Determine the absorbances,  $A_{310}$  at 310 nm,  $A_{325}$  at 325 nm, and  $A_{334}$  at 334 nm, of the sample solution as directed under Ultraviolet-visible Spectrophotometry.

Units of vitamin A in 1 g of the sample

$$= \frac{A_{325}}{M} \times \frac{V}{100} \times f \times 1830$$

$$f = 6.815 - 2.555 \times \frac{A_{310}}{A_{325}} - 4.260 \times \frac{A_{334}}{A_{325}}$$

$A_{325}$ : Absorbance at 325 nm

$V$ : Total volume (mL) of the sample solution

$M$ : Amount (g) of sample in  $V$  mL of the sample solution

$f$ : Correction factor

1830: Conversion factor from specific absorbance of retinol alcohol to IU (Unit/g)

## 2.56 Determination of Specific Gravity and Density

The density  $\rho$  (g/mL or g/cm<sup>3</sup>) 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 stand-

ard substance. The relative density is also called the specific gravity.

The specific gravity,  $d'_t$ , means the ratio of the mass of the sample specimen at  $t'^\circ\text{C}$  to that of an equal volume of water (H<sub>2</sub>O) at  $t^\circ\text{C}$ . 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.

### 1. Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10 mL to 100 mL, having a ground-glass stopper fitted with a thermometer, and a side inlet-tube 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 sample solution, keeping them at a slightly lower temperature by 1°C to 3°C than the specified temperature  $t'^\circ\text{C}$ , 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 sample 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 sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass  $M_2$  at the specified temperature  $t^\circ\text{C}$ . The specific gravity  $d'_t$  can be calculated by use of the following equation.

$$d'_t = \frac{M_1 - M}{M_2 - M}$$

Further, when measurements for a sample solution and water are performed at the same temperature ( $t^\circ\text{C} = t'^\circ\text{C}$ ), the density of the sample solution at the temperature  $t'^\circ\text{C}$  ( $\rho'_T$ ) can be calculated from the measured specific gravity  $d'_t$  and the density of water at the temperature  $t'^\circ\text{C}$  ( $\rho'_{S1}$ ) indicated in Table 2.56-1 by using the following equation.

$$\rho'_T = \rho'_{S1} d'_t$$

### 2. Method 2. Measurement using a Sprengel-Ostwald pycnometer

A Sprengel-Ostwald pycnometer is a glass vessel with a capacity of usually 1 mL to 10 mL. As shown in Fig. 2.56-1, both ends are thick-walled fine tubes (inside diameter: 1 -

Table 2.56-1 Density of water

Temp. °C	Density g/mL						
0	0.999 84	11	0.999 61	21	0.997 99	31	0.995 34
1	0.999 90	12	0.999 50	22	0.997 77	32	0.995 03
2	0.999 94	13	0.999 38	23	0.997 54	33	0.994 70
3	0.999 96	14	0.999 24	24	0.997 30	34	0.994 37
4	0.999 97	15	0.999 10	25	0.997 04	35	0.994 03
5	0.999 96	16	0.998 94	26	0.996 78	36	0.993 68
6	0.999 94	17	0.998 77	27	0.996 51	37	0.993 33
7	0.999 90	18	0.998 60	28	0.996 23	38	0.992 97
8	0.999 85	19	0.998 41	29	0.995 94	39	0.992 59
9	0.999 78	20	0.998 20	30	0.995 65	40	0.992 22

\* 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<sup>3</sup> seriously.

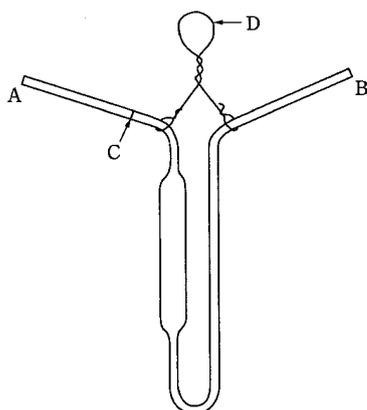


Fig. 2.56-1 Sprengel-Ostwald pycnometer

1.5 mm, outside diameter: 3 – 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 sample solution, which is at a lower temperature by  $3^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  than the specified temperature  $t^{\circ}\text{C}$ . Attach rubber tubing or a ground-glass tube to the end of A, and suck up the sample 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  $t^{\circ}\text{C}$  for about 15 minutes, and then, by attaching a piece of filter paper to the end of B, adjust the level of the sample 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  $t^{\circ}\text{C}$ , and note the mass  $M_2$ . Calculate the specific gravity  $d_t^t$ , according to the equation described in Method 1.

Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature ( $t^{\circ}\text{C} = t^{\circ}\text{C}$ ), the density of sample solution at temperature  $t^{\circ}\text{C}$  can be calculated by using the equation described in Method 1.

### 3. Method 3. Measurement using a hydrometer

Clean a hydrometer with ethanol (95) or diethyl 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  $t^{\circ}\text{C}$  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  $t^{\circ}\text{C}$  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 sample solution is performed at the same temperature ( $t^{\circ}\text{C} = t^{\circ}\text{C}$ ), at which the hydrometer is calibrated, the density of a sample solution at  $t^{\circ}\text{C}$ ,  $\rho_T^t$ , can be calculated by using the specific gravity  $d_t^t$  and the equation shown in Method 1.

### 4. Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter

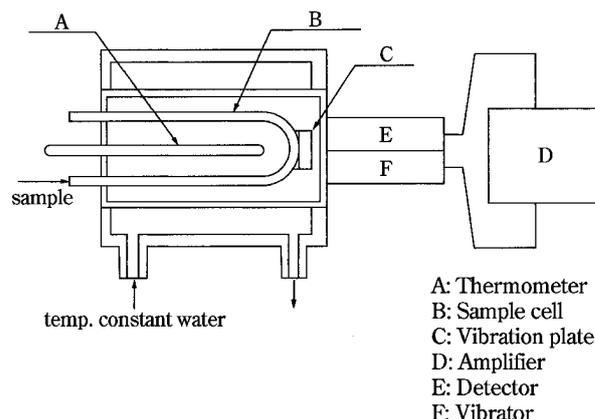


Fig. 2.56-2 Oscillator-type density meter

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:  $\rho_{S1}$ ,  $\rho_{S2}$ ) must be measured at a specified temperature  $t^{\circ}\text{C}$ , and the cell constant  $K_t$  ( $\text{g} \cdot \text{cm}^{-3} \text{s}^{-2}$ ) 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  $t^{\circ}\text{C}$ ,  $\rho_{S1}^t$ , is taken from Table 2.56-1, and that of dried air  $\rho_{S2}^t$  is calculated by using the following equation, where the pressure of dried air is at  $p$  kPa.

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

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  $t^{\circ}\text{C}$ ,  $\rho_T^t$ , 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  $t^{\circ}\text{C}$ ,  $\rho_{S1}^t$ , 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  $t^{\circ}\text{C}$  can be obtained by using the equation below, by introducing the density of water at a temperature  $t^{\circ}\text{C}$ ,  $\rho_{S1}^t$ , indicated in Table 2.56-1.

$$d_t^t = \frac{\rho_T^t}{\rho_{S1}^t}$$

#### 4.1. Apparatus

An oscillator-type density meter is usually composed of a glass tube cell of about 1 mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which ap-

plies 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 Fig. 2.56-2.

#### 4.2. Procedure

A sample cell, water, and a sample specimen are previously adjusted to a specified temperature  $t'^{\circ}\text{C}$ . 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  $\rho'_{S1}$ , and the cell constant  $K'_t$ , the density of the sample specimen  $\rho'_t$  can be obtained by use of the above equation. If necessary, the specific gravity of the sample specimen  $d'_t$  against water at a temperature  $t^{\circ}\text{C}$ , can be calculated by using the density of water  $\rho'_{S1}$  shown in Table 2.56-1.

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

## 2.57 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.

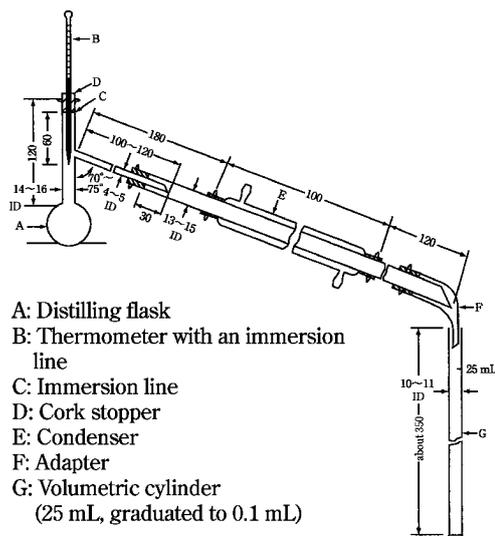
**1. Method 1** This method is applied to a sample for which the permissible range of boiling temperature is smaller than  $5^{\circ}\text{C}$ .

#### 1.1. Apparatus

Use the apparatus illustrated in Fig. 2.57-1.

#### 1.2. Procedure

Measure 25 mL of the sample, whose temperature is previously noted, using a volumetric cylinder G graduated in 0.1 mL, and transfer it to a distilling flask A of 50- to 60-mL 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



The figures are in mm.

Fig. 2.57-1

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-insulation materials in about 6 mm thickness), having an 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 4 to 5 mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than  $200^{\circ}\text{C}$  and at a rate of 3 to 4 mL per minute in the case of liquids whose boiling temperature is  $200^{\circ}\text{C}$  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  $80^{\circ}\text{C}$  are cooled to between  $10^{\circ}\text{C}$  and  $15^{\circ}\text{C}$  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.

**2. Method 2** This method is applied to the sample for which the permissible range of boiling temperature is  $5^{\circ}\text{C}$  or more.

#### 2.1. Apparatus

The same apparatus as described in Method 1 is used. However, use a 200-mL distilling flask A with a neck 18 to 24 mm in inside diameter having a delivery tube 5 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.

## 2.2. Procedure

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

## 2.58 X-Ray Powder Diffraction Method

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

♦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<sup>(1)</sup> 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. 2.58-1)

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

The wavelength  $\lambda$  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').  $\theta_{hkl}$  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

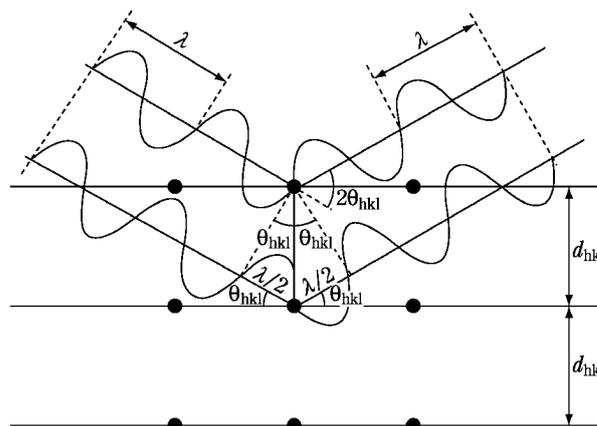
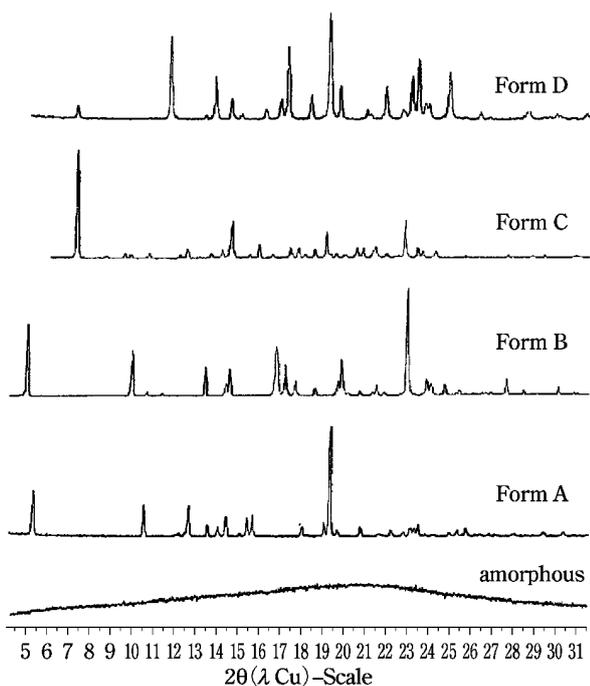


Fig. 2.58-1 Diffraction of X-rays by a crystal according to Bragg's law

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,  $\alpha$ ,  $\beta$  and  $\gamma$ . 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,  $\theta_{hkl}$ , associated with it (for a specific wavelength  $\lambda$ ).

A powder specimen is assumed to be polycrystalline so that at any angle  $\theta_{hkl}$  there are always crystallites in an orientation allowing diffraction according to Bragg's law<sup>(2)</sup>. 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  $2\theta$  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. 2.58-2.

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.



**Fig. 2.58-2** X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities are normalized)

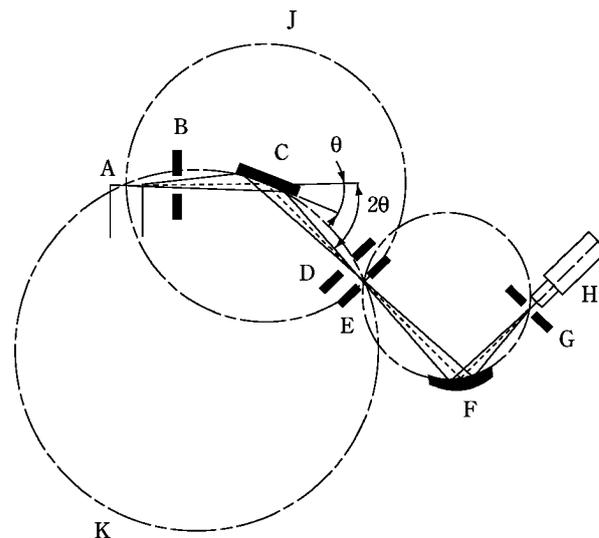
## 2. Instrument

### 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 required 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  $\theta/\theta$  geometry. For both geometries, the incident X-ray beam forms an angle  $\theta$  with the specimen surface plane and the diffracted X-ray beam forms an angle  $2\theta$  with the direction of the incident X-ray beam (an angle  $\theta$  with the specimen surface plane). The basic geometric arrangement represented in Fig. 2.58-3. The divergent beam of radiation from the X-ray tube (the so-called 'primary beam') passes through the parallel plate collimators



- 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. 2.58-3** Geometric arrangement of the Bragg-Brentano parafocusing geometry

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  $2\theta$  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 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  $K_{\alpha}$  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  $K_{\beta}$  filters, i.e. metal filters selected as having an absorption edge between the  $K_{\alpha}$  and  $K_{\beta}$  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.  $K_{\alpha}$  and  $K_{\beta}$ ) at different angles, so that only one of them may be selected to enter into the detector. It is even possible to separate  $K_{\alpha 1}$  and  $K_{\alpha 2}$  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  $K_{\alpha}$  and  $K_{\beta}$  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<sup>(9)</sup>. 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  $50\ \mu\text{m}$  will provide satisfactory results in phase identification. However, excessive milling (crystallite sizes less than approximately  $0.5\ \mu\text{m}$ ) 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 particles 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  $2\theta$  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  $2\theta$ -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  $I_{\text{norm}}$ , a so-called  $(d, I_{\text{norm}})$ -list extracted from the pattern, is the crystallographic fingerprint of the material, and can be compared to  $(d, I_{\text{norm}})$ -lists of single-phase samples compiled in databases.

For most organic crystals, when using Cu  $K_{\alpha}$  radiation, it is appropriate to record the diffraction pattern in a  $2\theta$ -range from as near  $0^{\circ}$  as possible to at least  $40^{\circ}$ . The agreement in the  $2\theta$ -diffraction angles between specimen and reference is within  $0.2^{\circ}$  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  $2\theta$  positions of greater than  $0.2^{\circ}$  is not unexpected. As such, peak position variances such as  $0.2^{\circ}$  are not applicable to these materials. For other types of samples (e.g. inorganic salts), it may be necessary to extend the  $2\theta$ -region scanned to well beyond  $40^{\circ}$ . 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<sup>(4)</sup>, 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  $a$ :

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

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 are not known, the Pawley or least squares methods can be used.

## 2.59 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 car-

bon. The other method is to remove inorganic carbon from the test water, then to measure the amount of remaining organic carbon.

### 1. 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 than 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 decomposition process or separates carbon dioxide from 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.

### 2. Reagents and standard solutions

(i) 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.

(ii) 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°C 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.

(iii) 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°C and 600°C 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.

(iv) 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.

(v) Gas for removing inorganic carbon or carrier gas:

Nitrogen, oxygen, or other gases that can be used for the same purpose.

(vi) 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.

### 3. Apparatus

(i) 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.

(ii) 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.

### 4. 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.

#### 4.1. 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.

#### 4.2. 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.

## 2.60 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 substance 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 can not be readily pulverized, and Method 3 to petrolatum.

Unless otherwise specified, measurement is performed by Method 1.

### 1. Method 1

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

#### 1.1. Apparatus

Use the apparatus illustrated in the Fig. 2.60-1.

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

(i) Bath fluid: Usually use clear silicone oil having a viscosity of 50 to 100 mm<sup>2</sup>/s at an ordinary temperature.

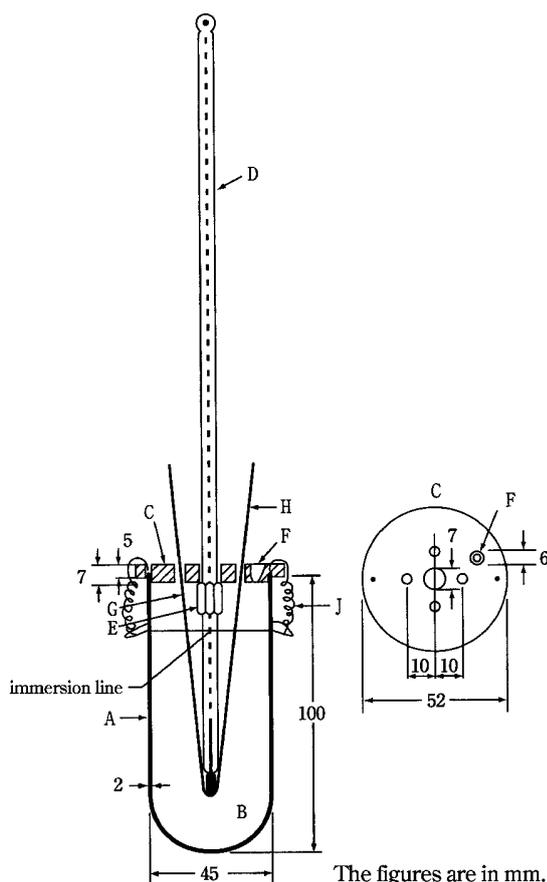
(ii) Thermometer with an immersion line: There are six types of thermometers, Type 1—Type 6, which are specified by an appropriate measuring temperature range. For melting points lower than 50°C, use a thermometer Type 1; for 40°C to 100°C, Type 2; for 90°C to 150°C, Type 3; for 140°C to 200°C, Type 4; for 190°C to 250°C, Type 5; for 240°C to 320°C, Type 6.

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

#### 1.2. Procedure

Pulverize 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 specified in the monograph before measurement. Place the sample in a dried capillary tube H, and pack it tightly so as to form a layer about 2.5–3.5 mm high by dropping the capillary 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 thermometer D in the bath with the immersion line at the same level as



- A: Heating vessel of 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
- J: Spring for fastening Teflon stopper

Fig. 2.60-1 Melting point determination apparatus

the meniscus of the bath fluid, and insert capillary tube H into a coil spring G so that the packed sample is placed in a position corresponding to the center of the mercury bulb of the thermometer D. Continue heating to raise the temperature at a rate of approximately 3°C per minute until the temperature rises to 5°C below the expected melting point, then carefully regulate the rate of temperature increase to 1°C per minute.

Read the thermometer indication of the instantaneous temperature at which the sample liquefies completely and no solid is detectable in the capillary, and designate the indicated temperature as the melting point of the sample specimen.

#### 1.2.1. System suitability test

Confirmation of the system suitability of the apparatus should be done periodically by using the Melting Point 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 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  $MP_f \pm 0.5^\circ\text{C}$  in the case of vanillin and acetanilide, within  $MP_f \pm 0.8^\circ\text{C}$  in the case of acetophenetidine and sulfanilamide, and within  $MP_f \pm 1.0^\circ\text{C}$  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.

## 2. Method 2

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

### 2.1. 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.

### 2.2. Procedure

Carefully melt the sample at as low a temperature as possible, and, taking care to prevent bubbles, introduce it into a capillary tube to a height of about 10 mm. Allow the capillary containing the sample to stand for 24 hours at below 10°C, or for at least 1 hour in contact with ice, holding the capillary so that the sample can not flow out. Then attach the capillary to the thermometer by means of a rubber band so that the absorbed sample is located at a position corresponding to the center of the mercury bulb. Adjust the capillary tube in a water-containing beaker to such a position that the lower edge of the sample is located 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 temperature increase to 1°C per minute. The temperature at which the sample begins floating in the capillary is taken as the melting point of the sample specimen.

## 3. Method 3

This method is applied to petrolatums.

### 3.1. 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.

### 3.2. Procedure

Melt the sample slowly by heating, with thorough stirring, until the temperature reaches 90–92°C. Discontinue the heating, and allow the sample to cool to 8–10°C above the expected melting point. Chill the bulb of the thermometer to 5°C, wipe and dry, and, while still cold, stick half of the thermometer bulb into the melted sample. Withdraw it immediately, hold vertically, cool until the attached sample becomes turbid, then dip the sample-bearing bulb for 5 minutes in water having a temperature below 16°C. Next, fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is located 15 mm above the bottom. Suspend the test tube in a water-containing beaker held at a temperature about 16°C, and raise the temperature of the water bath to 30°C at a rate of 2°C per minute, then continue heating carefully at a rate of 1°C per minute until it reaches the melting point. Read the thermometer indication of the instantaneous temperature at which the first drop of the sample leaves the thermometer. If the variations between three repeated determinations are not more than 1°C, take the average of the three as the melting point. If any variation is greater than 1°C, make two additional measurements, and take the average of the five as the melting point.

## 3. Powder Property Determinations

### 3.01 Determination of Bulk and Tapped Densities

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

♦Determination of Bulk and Tapped Densities is a method to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant.♦

#### 1. Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/mL) although the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m<sup>3</sup>) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm<sup>3</sup>).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest distur-

bance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may have been passed through a sieve into a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or a measuring vessel (Method 3). Method 1 and Method 3 are favoured.

#### 1.1. Method 1: Measurement in a graduated cylinder

##### 1.1.1. Procedure

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample (*m*) weighed with 0.1 per cent accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (*V*<sub>0</sub>) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula *m/V*<sub>0</sub>. Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 mL and 100 mL, a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

#### 1.2. Method 2: Measurement in a volumeter

##### 1.2.1. Apparatus

The apparatus<sup>(1)</sup> (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baf-

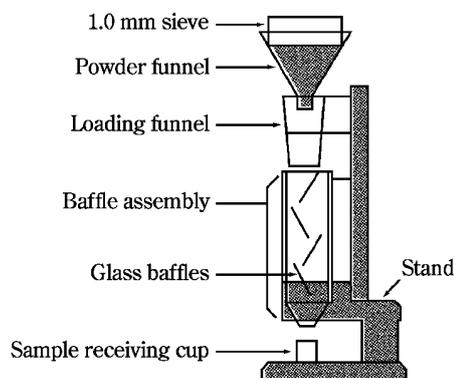


Fig. 3.01-1 Volumeter

the box is a funnel that collects the powder and allows it to pour into a cup mounted directly below it. The cup may be cylindrical ( $25.00 \pm 0.05$  mL volume with an inside diameter of  $30.00 \pm 2.00$  mm) or cubical ( $16.39 \pm 0.20$  mL volume with inside dimensions of  $25.4 \pm 0.076$  mm).

### 1.2.2. Procedure

Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of  $25 \text{ cm}^3$  of powder with the cubical cup and  $35 \text{ cm}^3$  of powder with the cylindrical cup. Carefully, scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the side of the cup and determine the mass ( $m$ ) of the powder to the nearest 0.1 per cent. Calculate the bulk density in g per mL by the formula  $m/V_0$  in which  $V_0$  is the volume of the cup and record the average of 3 determinations using 3 different powder samples.

### 1.3. Method 3: Measurement in a vessel

#### 1.3.1. Apparatus

The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Fig. 3.01-2.

#### 1.3.2. Procedure

Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method 2. Determine the mass ( $m_0$ ) of the powder to the nearest 0.1 per cent by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula  $m_0/100$  and record the average of 3 determinations using 3 different powder samples.

## 2. Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance by either of 3 methods as described below. Devices that

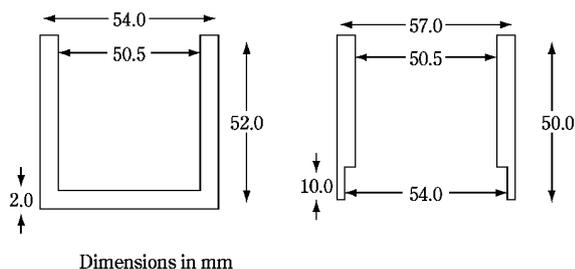


Fig. 3.01-2 Measuring vessel (left) and cap (right)

rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

## 2.1. Method 1

### 2.1.1. Apparatus

The apparatus (Fig. 3.01-3) consists of the following:

- (i) a 250 mL graduated cylinder (readable to 2 mL) with a mass of  $220 \pm 44$  g,
- (ii) a settling apparatus capable of producing, in 1 min, either nominally  $250 \pm 15$  taps from a height of  $3 \pm 0.2$  mm, or nominally  $300 \pm 15$  taps from a height of  $14 \pm 2$  mm. The support for the graduated cylinder, with its holder, has a mass of  $450 \pm 10$  g.

### 2.1.2. Procedure

Proceed as described above for the determination of the bulk volume ( $V_0$ ).

Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes  $V_{10}$ ,  $V_{500}$  and  $V_{1250}$  to the nearest graduated unit. If the difference between  $V_{500}$  and  $V_{1250}$  is less than or equal to 2 mL,  $V_{1250}$  is the tapped volume. If the difference between  $V_{500}$  and  $V_{1250}$  exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula  $m/V_f$  in which  $V_f$  is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing  $130 \pm 16$  g and mounted on a holder weighing  $240 \pm 12$  g. The modified test conditions are specified in the expression of the results.

## 2.2. Method 2

### 2.2.1. Procedure

Proceed as directed under Method 1 except that the mechanical tester provides a fixed drop of  $3 \pm 0.2$  mm at a nominal rate of 250 taps per minute.

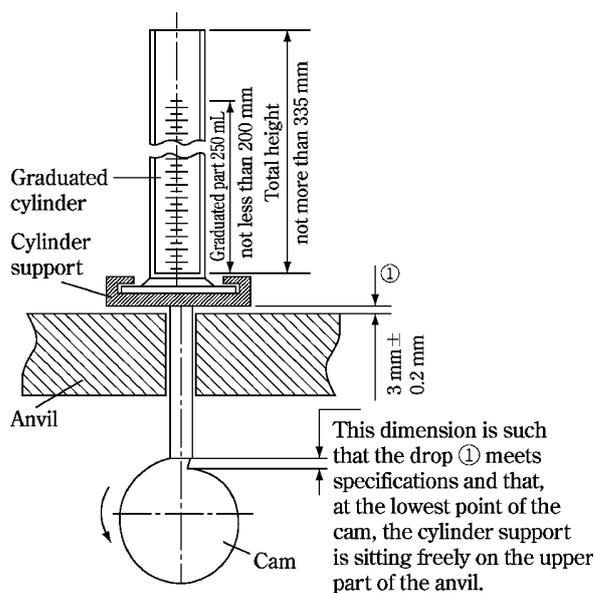


Fig. 3.01-3

### 2.3. Method 3

#### 2.3.1. Procedure

Proceed as described in the method for measuring the bulk density using the measuring vessel equipped with the cap shown in Fig. 3.01-2. The measuring vessel with the cap is lifted 50-60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrape excess powder from the top of the measuring vessel as described in Method 3 for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the 2 masses obtained after 200 and 400 taps exceeds 2 per cent, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2 per cent. Calculate the tapped density (g/mL) using the formula  $m_f/100$  where  $m_f$  is the mass of powder in the measuring vessel. Record the average of 3 determinations using 3 different powder samples. The test conditions including tapping height are specified in the expression of the results.

### 3. Measures of powder compressibility

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the Compressibility Index or the Hausner Ratio.

The Compressibility Index and Hausner Ratio are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio.

Compressibility Index:

$$100 (V_0 - V_f)/V_0$$

$V_0$ : unsettled apparent volume

$V_f$ : final tapped volume

Hausner Ratio:

$$V_0/V_f$$

Depending on the material, the compressibility index can be determined using  $V_{10}$  instead of  $V_0$ . If  $V_{10}$  is used, it is clearly stated in the results.

<sup>(1)</sup> The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.

## 3.02 Specific Surface Area by Gas Adsorption

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are

not harmonized are marked with symbols (♦ ♦).

♦The specific surface area determination method is a method to determine specific surface area (the total surface area of powder per unit mass) of a pharmaceutical powder sample by using gas adsorption method.♦ The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

### 1. Measurements

#### 1.1. Multi-point measurement

When the gas is physically adsorbed by the powder sample, the following relationship (Brunauer, Emmett and Teller (BET) adsorption isotherm) holds when the relative pressure ( $P/P_0$ ) is in the range of 0.05 to 0.30 for pressure  $P$  of the adsorbate gas in equilibrium for the volume of gas adsorbed,  $V_a$ .

$$\frac{1}{[V_a\{(P_0/P) - 1\}]} = \{(C - 1)/V_m C\} \times (P/P_0) + (1/V_m C) \quad (1)$$

$P$ : Partial vapour pressure of adsorbate gas in equilibrium with the surface at  $-195.8^\circ\text{C}$  (b.p. of liquid nitrogen), in pascals

$P_0$ : Saturated pressure of adsorbate gas, in pascals

$V_a$ : Volume of gas adsorbed at standard temperature and pressure (STP) [ $0^\circ\text{C}$  and atmospheric pressure ( $1.013 \times 10^5$  Pa)], in milliliters

$V_m$ : Volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters

$C$ : Dimensionless constant that is related to the enthalpy of adsorption of adsorbate gas on the powder sample

A value of  $V_a$  is measured at each of not less than 3 values of  $P/P_0$ . Then the BET value,  $1/[V_a\{(P_0/P) - 1\}]$ , is plotted against  $P/P_0$  according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient,  $r$ , of the linear regression is not less than 0.9975; that is,  $r^2$  is not less than 0.995. From the resulting linear plot, the slope, which is equal to  $(C - 1)/V_m C$ , and the intercept, which is equal to  $1/(V_m C)$ , are evaluated by linear regression analysis. From these values,  $V_m$  is calculated as  $1/(\text{slope} + \text{intercept})$ , while  $C$  is calculated as  $(\text{slope}/\text{intercept}) + 1$ . From the value of  $V_m$  so determined, the specific surface area,  $S$ , in  $\text{m}^2\text{g}^{-1}$ , is calculated by the equation:

$$S = (V_m N a)/(m \times 22,400) \quad (2)$$

$N$ : Avogadro constant ( $6.022 \times 10^{23} \text{ mol}^{-1}$ ),

$a$ : Effective cross-sectional area of one adsorbate molecule, in square meters ( $0.162 \times 10^{-18} \text{ m}^2$  for nitrogen and  $0.195 \times 10^{-18} \text{ m}^2$  for krypton)

$m$ : Mass of test powder, in grams

22,400: Volume, in milliliters, occupied by one mole of the adsorbate gas at STP allowing for minor departures from the ideal

A minimum of 3 data points is required. Additional meas-

measurements may be carried out, especially when non-linearity is obtained at a  $P/P_0$  value close to 0.3. Because non-linearity is often obtained at a  $P/P_0$  value below 0.05, values in this region are not recommended. The test for linearity, the treatment of the data, and the calculation of the specific surface area of the sample are described above.

### 1.2. Single-point measurement

Normally, at least 3 measurements of  $V_a$  each at different values of  $P/P_0$  are required for the determination of specific surface area by the dynamic flow gas adsorption technique (*Method I*) or by volumetric gas adsorption (*Method II*). However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of  $V_a$  measured at a single value of  $P/P_0$  such as 0.300 (corresponding to 0.300 mole of nitrogen or 0.001038 mole fraction of krypton), using the following equation for calculating  $V_m$ :

$$V_m = V_a \{1 - (P/P_0)\} \quad (3)$$

The single-point method may be employed directly for a series of powder samples of a given material for which the material constant  $C$  is much greater than unity. These circumstances may be verified by comparing values of specific surface area determined by the single-point method with that determined by the multiple-point method for the series of powder samples. Close similarity between the single-point values and multiple-point values suggests that  $1/C$  approaches zero. The single-point method may be employed indirectly for a series of very similar powder samples of a given material for which the material constant  $C$  is not infinite but may be assumed to be invariant. Under these circumstances, the error associated with the single-point method can be reduced or eliminated by using the multiple-point method to evaluate  $C$  for one of the samples of the series from the BET plot, from which  $C$  is calculated as  $(1 + \text{slope}/\text{intercept})$ . Then  $V_m$  is calculated from the single value of  $V_a$  measured at a single value of  $P/P_0$  by the equation:

$$V_m = V_a \{(P_0/P) - 1\} [(1/C) + \{(C - 1)/C\} \times (P/P_0)] \quad (4)$$

### 2. Sample preparation

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface during storage and handling. If outgassing is not achieved, the specific surface area may be reduced or may be variable because some parts of surface area are covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials. The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure and time should be so chosen that the original surface of the solid is reproduced as closely as possible.

Outgassing of many substances is often achieved by applying a vacuum, by purging the sample in a flowing stream of a non-reactive, dry gas, or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised

when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

The standard technique is the adsorption of nitrogen at liquid nitrogen temperature.

For powders of low specific surface area ( $<0.2 \text{ m}^2\text{g}^{-1}$ ) the proportion adsorbed is low. In such cases the use of krypton at liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error. All gases used must be free from moisture.

Accurately weigh a quantity of the test powder such that the total surface of the sample is at least  $1 \text{ m}^2$  when the adsorbate is nitrogen and  $0.5 \text{ m}^2$  when the adsorbate is krypton. Lower quantities of sample may be used after appropriate validation.

Because the amount of gas adsorbed under a given pressure tends to increase on decreasing the temperature, adsorption measurements are usually made at a low temperature. Measurement is performed at  $-195.8^\circ\text{C}$ , the boiling point of liquid nitrogen.

Adsorption of gas should be measured either by Method I or Method II.

## 3. Methods

### 3.1. Method 1: The dynamic flow method

In the dynamic flow method (see Fig. 3.02-1), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions. A minimum of 3 mixtures of

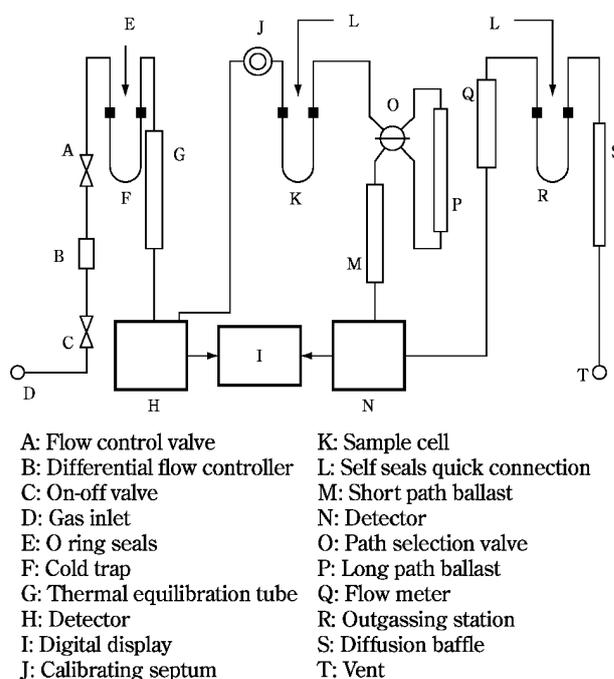


Fig. 3.02-1 Schematic diagram of the dynamic flow method apparatus

the appropriate adsorbate gas with helium are required within the  $P/P_0$  range 0.05 to 0.30.

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of 3 data points within the recommended range of 0.05 to 0.30 for  $P/P_0$  is to be determined.

A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again through the thermal conductivity cell and then to a recording potentiometer. Immerse the sample cell in liquid nitrogen, then the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

Remove from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak.

Since this is better defined than the adsorption peak, it is the one used for the determination.

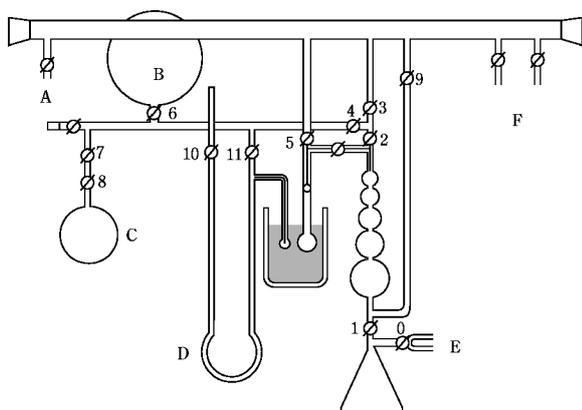
To effect the calibration, inject a known quantity of adsorbate into the system, sufficient to give a peak of similar magnitude to the desorption peak and obtain the proportion of gas volume per unit peak area.

Use a nitrogen/helium mixture for a single-point determination and several such mixtures or premixing 2 streams of gas for a multiple-point determination. Calculation is essentially the same as for the volumetric method.

### 3.2. Method 2: The volumetric method

In the volumetric method (see Fig. 3.02-2), the recommended adsorbate gas is nitrogen is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure,  $P$ , of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Since only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.



A: Vacuum gauge      D: Vapour pressure manometer  
B: Nitrogen reservoir      E: Vacuum air  
C: Helium reservoir      F: To cold traps and vacuum pumps

Fig. 3.02-2 Schematic diagram of the volumetric method apparatus

Admit a small amount of dry nitrogen into the sample tube to prevent contamination of the clean surface, remove the sample tube, insert the stopper, and weigh it. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure (e.g. between 2 Pa and 10 Pa). Alternately, some instruments operate by evacuating to a defined rate of pressure change (e.g. less than 13 Pa/30 s) and holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a non-adsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements, that is, by means of reference and sample tubes connected by a differential transducer.

Raise a Dewar vessel containing liquid nitrogen at  $-195.8^{\circ}\text{C}$  up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed,  $V_a$ . For multipoint measurements, repeat the measurement of  $V_a$  at successively higher  $P/P_0$  values. When nitrogen is used as the adsorbate gas,  $P/P_0$  values of 0.10, 0.20, and 0.30 are often suitable.

### 4. Reference materials

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as  $\alpha$ -alumina for specific surface area determination, which should have a specific surface area similar to that of the sample to be examined.

## 3.03 Powder Particle Density Determination

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the test that are not harmonized are marked with symbols ( $\blacklozenge$   $\blacklozenge$ ).

Powder Particle Density Determination is  $\blacklozenge$  a method to determine particle density of powdered pharmaceutical drugs or raw materials of drugs  $\blacklozenge$ , and generally performed using a gas displacement pycnometer. The gas pycnometric density is determined by measuring the volume occupied by a known mass of powder which is equivalent to the volume of gas displaced by the powder using a gas displacement pycnometer. In gas pycnometric density measurements, the volume determined excludes the volume occupied by open pores; however, it includes the volume occupied by sealed pores or pores inaccessible to the gas.

Usually, helium is used as a test gas due to its high diffusivity into small open pores. If gases other than helium are used, different values would be obtained, since the penetration of the gas is dependent on the size of the pore as well as the cross-sectional area of the gas.

The measured density is a volume weighted average of the densities of individual powder particles. It is called the particle density, distinct from the true density of solid or the bulk density of powder. The density of solids are expressed in

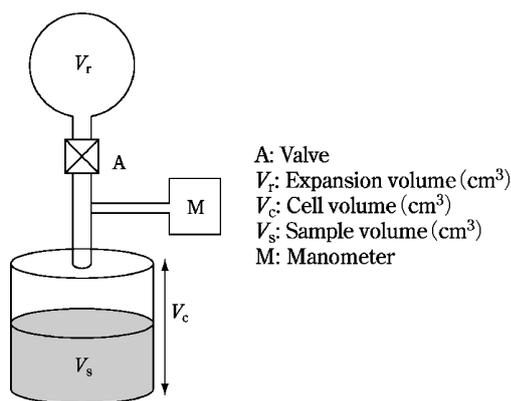


Fig. 3.03-1 Schematic diagram of a gas pycnometer

grams per cubic centimeter (g/cm<sup>3</sup>), although the international unit is the kilogram per cubic meter (1 g/cm<sup>3</sup> = 1000 kg/m<sup>3</sup>).

### 1. Apparatus

The schematic diagram of particle density apparatus for gas displacement pycnometric measurement is shown in Fig. 3.03-1. The apparatus consists of a test cell in which the sample is placed, an expansion cell and a manometer (M). The test cell, with an empty cell volume ( $V_c$ ), is connected through a valve (A) to an expansion cell, with a volume ( $V_r$ ).

Generally, helium is used as the measurement gas. The apparatus has to be equipped with a system capable of pressuring the test cell to the defined pressure ( $P$ ) through the manometer.

### 2. Calibration of apparatus

The volumes of the test cell ( $V_c$ ) and the expansion cell ( $V_r$ ) must be accurately determined to the nearest 0.001 cm<sup>3</sup>, and to assure accuracy of the results of volume obtained, calibration of the apparatus is carried out as follows using a calibration ball of known volume for particle density measurement. The final pressures ( $P_f$ ) are determined for the initial empty test cell followed by the test cell placed with the calibration ball for particle density measurement in accordance with the procedures, and  $V_c$  and  $V_r$  are calculated using the equation described in the section of Procedure. Calculation can be made taking into account that the sample volume ( $V_s$ ) is zero in the first run.

### 3. Procedure

The gas pycnometric density measurement is performed at a temperature between 15°C and 30°C and must not vary by more than 2°C during the course of measurement.

Volatile contaminants in the powder are removed by degassing the powder under a constant purge of helium prior to the measurement. Occasionally, powders may have to be degassed under vacuum. Because volatiles may be evolved during the measurement, weighing of the sample is done after the pycnometric measurement of volume.

Weigh the mass of the test cell and record it. After weighing out the amount of the sample as described in the individual monograph and placing it in the test cell, seal the cell in the pycnometer.

Open the valve (A) which connects the expansion cell with the test cell, confirm with the manometer (M) that the pres-

sure inside the system is stable, and then read the system reference pressure ( $P_r$ ). Secondly, close the valve that connects to the two cells, and introduce the measurement gas into the test cell to achieve positive pressure. Confirm with the manometer that the pressure inside the system is stable, and then read the initial pressure ( $P_i$ ). Open the valve to connect the test cell with the expansion cell. After confirming that the indicator of the manometer is stable, read the final pressure ( $P_f$ ), and calculate the sample volume ( $V_s$ ) with the following equation.

$$V_s = V_c - \frac{V_r}{\frac{P_i - P_r}{P_f - P_r} - 1}$$

$V_r$ : Expansion volume (cm<sup>3</sup>)

$V_c$ : Cell volume (cm<sup>3</sup>)

$V_s$ : Sample volume (cm<sup>3</sup>)

$P_i$ : Initial pressure (kPa)

$P_f$ : Final pressure (kPa)

$P_r$ : System reference pressure (kPa)

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume agree to within 0.2%, and calculate the mean of sample volumes ( $V_s$ ). Finally, unload the test cell, weigh the mass of the test cell, and calculate the final sample mass ( $m$ ) by deducting the empty cell mass from the test cell mass. The powder particle density  $\rho$  is calculated by the following equation:

$$\rho = m/V_s$$

$\rho$ : Powder particle density in g/cm<sup>3</sup>,

$m$ : Final sample mass in g,

$V_s$ : Sample volume in cm<sup>3</sup>

If the pycnometer differs in operation or construction from the one shown in Fig. 3.03-1, follow the instructions of the manufacturer of the pycnometer. The sample conditioning is indicated with the results. For example, indicate whether the sample was tested as is or dried under specific conditions such as those described for loss on drying.

## 3.04 Particle Size Determination

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

♦ Particle Size Determination is a method to determine directly or indirectly morphological appearance, shape, size and its distribution of powdered pharmaceutical drugs and excipients to examine their micromeritic properties. Optical microscopy and analytical sieving method may be used depending on the measuring purpose and the properties of test specimen. ♦

### 1. Method 1. Optical Microscopy

♦ The optical microscopy is used to observe the morphological appearance and shape of individual particle either directly with the naked eye or by using a microscopic photograph, in order to measure the particle size. The particle size distribution can also be determined by this method. It is also possible with this method to measure the size of the individ-

ual particle even when different kinds of particles mingle if they are optically distinguishable. Data processing techniques, such as image analysis, can be useful for determining the particle size distribution. ♦

This method for particle characterization can generally be applied to particles 1  $\mu\text{m}$  and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

### 1.1. Apparatus

Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives and are preferable with apochromats and are required for appropriate color rendition in photomicrography. Condensers corrected for at least spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the condition of use; this is affected by the actual aperture of the condenser diaphragm and the presence of immersion oils.

#### 1.1.1. Adjustment

The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

##### 1.1.1.1. Illumination

A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

##### 1.1.1.2. Visual Characterization

The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made.

♦When the particle size is measured, an ocular micrometer is inserted at the position of the ocular diaphragm, and a calibrated stage micrometer is placed at the center of the microscope stage and fixed in place. The ocular is attached to the lens barrel and adjusted to the focus point of the stage

micrometer scale. Then, the distance between the scales of the two micrometers is determined, and the sample size equivalent 1 division of the ocular scale is calculated using the following formula:

The particle size equivalent 1 division on the ocular scale ( $\mu\text{m}$ ) = Length on the stage micrometer ( $\mu\text{m}$ )/Number of scale divisions on the ocular micrometer

The stage micrometer is removed and the test specimen is placed on the microscope stage. After adjusting the focus, the particle sizes are determined from the number of scale divisions read through the ocular. ♦

Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

### 1.1.1.3. Photographic Characterization

If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photographic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

### 1.2. Preparation of the Mount

The mounting medium will vary according to the physical properties of the test specimen. Sufficient, but not excessive, contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting medium must include a consideration of the analyte solubility.

### 1.3. Characterization

#### 1.3.1. Crystallinity Characterization

The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

#### 1.3.2. Limit Test of Particle Size by Microscopy

Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10  $\mu\text{g}$  of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.

### 1.3.3. Particle Size Characterization

The measurement of particle size varies in complexity depending on the shape of the particle and the number of particles characterized must be sufficient to insure an acceptable level of uncertainty in the measured parameters<sup>1)</sup>. For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see Fig. 3.04-1).

(i) Feret's Diameter: The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.

(ii) Martin's Diameter: The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.

(iii) Projected area Diameter: The diameter of a circle that has the same projected area as the particle.

(iv) Length: The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.

(v) Width: The longest dimension of the particle measured at right angles to the length.

### 1.3.4. Particle Shape Characterization

For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the powder should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see Fig. 3.04-2).

(i) Acicular: Slender, needle-like particle of similar width

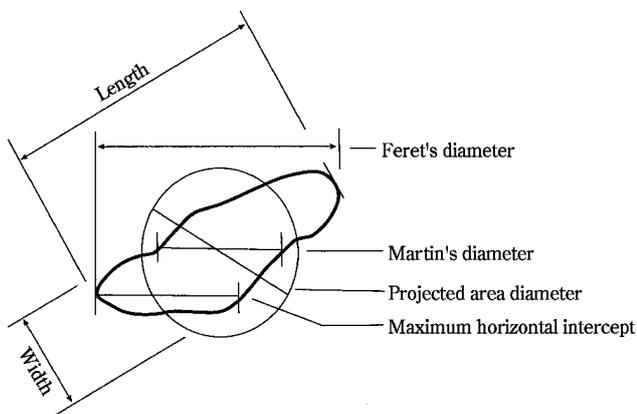


Fig. 3.04-1 Commonly used measurements of particle size

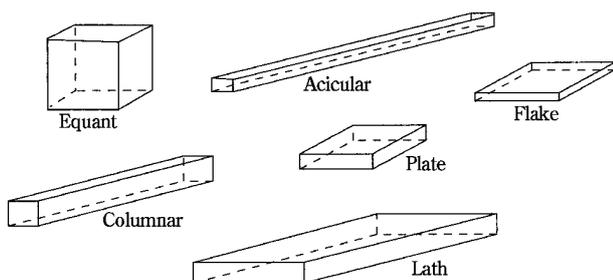


Fig. 3.04-2 Commonly used descriptions of particle shape

and thickness.

(ii) Columnar: Long, thin particle with a width and thickness that are greater than those of an acicular particle.

(iii) Flake: Thin, flat particle of similar length and width.

(iv) Plate: Flat particles of similar length and width but with greater thickness than flakes.

(v) Lath: Long, thin, and blade-like particle.

(vi) Equant: Particles of similar length, width, and thickness; both cubical and spherical particles are included.

### 1.3.5. General Observations

A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated.

This degree of association may be described by the following terms.

(i) Lamellar: Stacked plates.

(ii) Aggregate: Mass of adhered particles.

(iii) Agglomerate: Fused or cemented particles.

(iv) Conglomerate: Mixture of two or more types of particles.

(v) Spherulite: Radial cluster.

(vi) Drusy: Particle covered with tiny particles.

Particle condition may be described by the following terms.

(i) Edges: Angular, rounded, smooth, sharp, fractured.

(ii) Optical: Color (using proper color balancing filters), transparent, translucent, opaque.

(iii) Defects: Occlusions, inclusions.

Surface characteristics may be described by the following terms.

(i) Cracked: Partial split, break, or fissure.

(ii) Smooth: Free of irregularities, roughness, or projections.

(iii) Porous: Having openings or passageways.

(iv) Rough: Bumpy, uneven, not smooth.

(v) Pitted: Small indentations.

## 2. Method 2. Analytical Sieving Method

♦The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes. "Powder" here means a gathering of numerous solid particles. ♦

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about  $75 \mu\text{m}$ . For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than  $75 \mu\text{m}$  where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades

of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

Estimate the particle size distribution as described under *Dry Sieving Method*, unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below 75  $\mu\text{m}$ ), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

Principles of Analytical Sieving: Analytical test sieves are constructed from a woven-wire mesh, which is of simple weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75  $\mu\text{m}$ . The size parameter involved in determining particle size distribution by analytical sieving is the length of the size of the minimum square aperture through which the particle will pass.

## 2.1. Procedure

### 2.1.1. Test Sieves

Test sieves suitable for pharmacopoeial tests conform to the most current edition of International Organisation for Standardization (ISO) Specification ISO 3310-1; Test sieves—Technical requirements and testing (see Table 3.04-1).

Unless otherwise specified in the monograph, use those ISO sieves listed in the Table 3.04-1 as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having

a  $\sqrt{2}$  progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Mesh numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable non-reactive wire.

#### 2.1.1.1. Calibration of test sieves

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1<sup>2)</sup>. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850  $\mu\text{m}$ , Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

#### 2.1.1.2. Cleaning Test Sieves

Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

#### 2.1.2. Test Specimen

If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200 mm diameter. For 76 mm sieves the amount of material that can be accommodated is approximately 1/7<sup>th</sup> that which can be accommodated on a 200 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

#### 2.1.3. Agitation Methods

Several different sieve and powder agitation devices are

Table 3.04-1. Sizes of Standard Sieve Series in Range of Interest

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
Principal sizes	Supplementary sizes					
R 20/3	R 20	R 40/3				
11.20 mm	11.20 mm 10.00 mm	11.20 mm 9.50 mm			11200	
8.00 mm	9.00 mm 8.00 mm 7.10 mm	8.00 mm 6.70 mm				
5.60 mm	6.30 mm 5.60 mm 5.00 mm	5.60 mm 4.75 mm			5600	3.5
4.00 mm	4.50 mm 4.00 mm 3.55 mm	4.00 mm 3.35 mm	5	4000	4000	4.7
2.80 mm	3.15 mm 2.80 mm 2.50 mm	2.80 mm 2.36 mm	6 7			5.5
2.00 mm	2.24 mm 2.00 mm 1.80 mm	2.00 mm 1.70 mm	8 10	2800 2000	2800 2000	7.5 8.6
1.40 mm	1.60 mm 1.40 mm 1.25 mm	1.40 mm 1.18 mm	12 14		1400	10 12
1.00 mm	1.12 mm 1.00 mm 900 μm	1.00 mm 850 μm	16 18	1400 1000	1400 1000	14 16
710 μm	800 μm 710 μm 630 μm	710 μm 600 μm	20 25		710	18 22
500 μm	560 μm 500 μm 450 μm	500 μm 425 μm	30 35	710 500	710 500	26 30
355 μm	400 μm 355 μm 315 μm	355 μm 300 μm	40 45		355	36 42
250 μm	280 μm 250 μm 224 μm	250 μm 212 μm	50 60	355 250		50 60
180 μm	200 μm 180 μm 160 μm	180 μm 150 μm	70 80		250 180	70 83
125 μm	140 μm 125 μm 112 μm	125 μm 106 μm	100 120	180 125	180 125	100 119
90 μm	100 μm 90 μm 80 μm	90 μm 75 μm	140 170			140 166
63 μm	71 μm 63 μm 56 μm	63 μm 53 μm	200 230	90 63	90 63	200 235
45 μm	50 μm 45 μm 40 μm	45 μm 38 μm	270 325			282 330
					45	391

commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

#### 2.1.4. Endpoint Determination

The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

### 2.2. Sieving Methods

#### 2.2.1. Mechanical Agitation (Dry Sieving Method)

Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and place the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, a different particle size analysis method should be used.

#### 2.2.2. Air Entrainment Methods (Air Jet and Sonic Shifter Sieving)

Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as *air jet* sieving. It uses the same general sieving methodology as that described

under the *Dry Sieving Method*, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the *sonic sifting* method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic shifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75  $\mu\text{m}$ ), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

### 2.3. Interpretation

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

<sup>1</sup>Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276.

<sup>2</sup>International Organization for Standardization (ISO) Specification ISO 3310-1; Test sieves-Technical requirements and testing

## 4. Biological Tests/Biochemical Tests/Microbial Tests

### 4.01 Bacterial Endotoxins Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia.

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using a lysate reagent prepared from blood corpuscle extracts of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are two types of techniques for this test: the gel-clot techniques, which are based on 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 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 one of these techniques for the test. In the event of doubt or dispute, the final decision is made based on the gel-clot techniques, unless otherwise indicated.

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

### 1. 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.

### 2. Preparation of Solutions

#### 2.1. Preparation of Standard Endotoxin Stock Solution

Prepare Standard Endotoxin Stock Solution by dissolving Endotoxin Reference Standard that has been calibrated to the current WHO International Standard for Endotoxin, using water for bacterial endotoxins test (BET). Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

#### 2.2. 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.

#### 2.3. Preparation of Sample Solutions

Unless otherwise specified, prepare sample solutions by dissolving or diluting drugs, using water for BET. If necessary, adjust the pH of the solution to be examined so that the pH of the mixture of the lysate TS and sample solution falls within the specified pH range for the lysate reagent to be used. This usually applies to a sample 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.

### 3. Determination of Maximum Valid Dilution

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample solution at which the endotoxin limit can be determined.

Determine the MVD from the following equation:

$$\text{MVD} = (\text{Endotoxin limit} \times \text{Concentration of sample solution}) / \lambda$$

Endotoxin limit:

The endotoxin limit for injections, defined on the basis of dose, equals  $K/M$ , where  $K$  is a threshold pyrogenic dose of endotoxin per kg body mass (EU/kg), and  $M$  is equal to the maximum dose of product per kg body mass. When the product is to be injected at frequent intervals or infused continuously,  $M$  is the maximum total dose administered in a single hour period.

Concentration of sample 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)

$\lambda$ : the labeled lysate 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

### 4. 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 (4.1.1.) and for interfering factors (4.1.2.) as described under Preparatory testing (4.1.).

#### 4.1. Preparatory testing

##### 4.1.1. Test for confirmation of labeled lysate reagent sensitivity

The labeled sensitivity of lysate 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 to be used.

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

Prepare standard solutions having four concentrations equivalent to  $2\lambda$ ,  $\lambda$ ,  $0.5\lambda$  and  $0.25\lambda$  by diluting the Standard Endotoxin Stock Solution with water for BET. Mix a volume of the lysate TS with an equal volume of one of the standard solutions (usually, 0.1 mL aliquots) in each test tube. When single test vials or ampoules containing lyophilized lysate are used, add solutions directly to the vial or ampoule.

Keep the tubes (or containers such as vials or ampoules) containing the reaction mixture usually at  $37 \pm 1^\circ\text{C}$  for  $60 \pm 2$  minutes, avoiding vibration. To test the integrity of the gel after incubation, invert each tube or container through approximately  $180^\circ$  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  $0.25\lambda$  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 of the four replicate series using the following formula:

Geometric Mean Endpoint Concentration =  $\text{antilog}(\Sigma e/f)$

$\Sigma e$  = 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

Table 4.01-1

Solution	Endotoxin Concentration/ Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A*1	0/Sample solution	—	—	—	4
B*2	2λ/Sample solution	Sample solution	1	2λ	4
			2	1λ	
			4	0.5λ	
			8	0.25λ	
C*3	2λ/Water for BET	Water for BET	1	2λ	2
			2	1λ	
			4	0.5λ	
			8	0.25λ	
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.

than 0.5 λ and not more than 2.0 λ, the labeled sensitivity is confirmed, and is used in tests performed with this lysate.

#### 4.1.2. Test for interfering factors

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

Prepare the solutions A, B, C and D according to Table 4.01-1, and test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure described in 4.1.1.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described in 4.1.1.

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

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the sample to be examined. Furthermore, interference of the sample solution or diluted sample 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.

#### 4.2. Limit test

This method tests whether or not a sample contains endotoxins greater than the endotoxin limit specified in the individual monograph based on the gel formation in the

Table 4.01-2

Solution	Endotoxin concentration/Solution to which endotoxin is added	Number of replicates
A*1	0/Sample solution	2
B*2	2λ/Sample solution	2
C*3	2λ/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 solution 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.

presence of endotoxins at a concentration of more than the labeled lysate sensitivity.

#### 4.2.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-2. Making these four solutions one set, test two replicates of the set. In preparing solutions A and B, use the sample solutions complying with 4.1.2.

Concerning the test conditions including the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under 4.1.1.

#### 4.2.2. Interpretation

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

When a negative result is found for both replicates of solution A, the sample complies with the Bacterial Endotoxins Test.

When a positive result is found for both replicates of solution A, the sample does not comply with the test.

When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the sample complies with the test if a negative result is found for both replicates of solution A. The sample does not comply with the test if a positive result is found for one or both replicates of solution A.

However, if the sample does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

#### 4.3. Quantitative Test

This method measures endotoxin concentrations of samples by determining an endpoint of gel formation.

#### 4.3.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-3. Making these four solutions one set, test two replicates of the set. When preparing solutions A and B, use sample solutions complying with 4.1.2. Concerning the test conditions, follow the procedure described in 4.1.1.

#### 4.3.2. 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 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 the sample solution is calcu-

Table 4.01-3

Solution	Endotoxin concentration/ Solution to which endotoxin is added	Diluent	Dilution factor	Endotoxin concentration	Number of replicates
A*1	0/Sample solution	Water for BET	1 2 4 8	— — — —	2
B*2	2λ/Sample solution	—	1	2λ	2
C*3	2λ/Water for BET	Water for BET	1 2 4 8	2λ 1λ 0.5λ 0.25λ	2
D*4	0/Water for BET	—	—	—	2

- \*1 Sample solutions 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 solution at the same dilution as the solution A diluted at the lowest dilution factor, containing standard endotoxin at a concentration of 2λ.
- \*3 Standard endotoxin solutions for confirmation of the labeled lysate sensitivity.
- \*4 Negative control. Water for BET only.

lated by multiplying the endpoint dilution factor by λ.

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

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

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

## 5. Photometric quantitative techniques

### 5.1. Turbidimetric technique

This technique measures the endotoxin concentrations of samples based on the measurement of turbidity change accompanying gel formation of the lysate TS. This technique is classified as either endpoint-turbidimetric or kinetic-turbidimetric.

The endpoint-turbidimetric 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-turbidimetric 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.

### 5.2. Chromogenic technique

This technique measures the endotoxin concentrations of sample solutions 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-chromogenic technique 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 is usually carried out at 37 ± 1°C.

### 5.3. Preparatory testing

To assure the precision and validity of the turbidimetric or chromogenic techniques, perform both Test for assurance of criteria for the standard curve (5.3.1.) and Test for interfering factors (5.3.2.), as indicated below.

#### 5.3.1. 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 of 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 the standard curve.

If 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 standard curve does not comply with the test, repeat the test after verifying the test conditions.

#### 5.3.2. Test for interfering factors

Prepare solutions A, B, C and D according to Table 4.01-4. Perform the test on these solutions following the optimal conditions for the lysate reagent used (with regard to volume of sample solution and lysate TS, volume ratio of sample 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.

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 employed, or it is less than the endotoxin detection limit of the lysate 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 sample 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,

Table 4.01-4

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of test tubes or wells
A* <sup>1</sup>	0	Sample solution	Not less than 2
B* <sup>2</sup>	Middle concentration of the standard curve	Sample solution	Not less than 2
C* <sup>3</sup>	At least 3 concentrations	Water for BET	Each not less than 2
D* <sup>4</sup>	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 Sample solution 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 solutions at the concentrations used in 5.3.1. (for the standard curve).

\*4 Negative control. Water for BET only.

the sample 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 sample solution or diluted sample 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.

#### 5.4. Quantitative test

##### 5.4.1. Procedure

Prepare solutions A, B, C and D according to Table 4.01-4, and follow the procedure described in 5.3.2.

##### 5.4.2. Calculation of endotoxin concentration

Calculate the mean endotoxin concentration of solution A using the standard curve generated with solution C. The test is valid when all 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 employed, or it is less than the endotoxin detection limit of the lysate employed.

##### 5.4.3. 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/mL, EU/mg, EU/mEq or EU/Unit) specified in the individual monograph.

## 4.02 Microbial Assay for Antibiotics

Microbial Assay for Antibiotics is a method to determine the antimicrobial potency of antibiotics based on their antimicrobial activities. There are three methods for this test: the cylinder-plate, perforated plate, and turbidimetric methods. The former two are based on the measurement of the size of the zones of microbial growth inhibition in a nutrient agar medium, and the turbidimetric method is based on the measurement of the inhibition of turbidity development in a fluid medium with microbial growth. Unless otherwise specified in the individual monograph, tests specified to be carried out by the cylinder-plate method may be conducted under the same test conditions using the perforated plate method instead. If necessary, first sterilize water, isotonic sodium chloride solution, buffer solutions, reagents, test solutions and essential parts of measuring instruments and appliances to be used for the test. In performing the test, precautions must be taken to prevent biohazard.

### 1. Cylinder-plate method

The cylinder-plate method is a method to determine the antimicrobial potency of the antibiotic to be tested, and is based on the measurement of the size of the zone of growth inhibition of a test organism by the use of cylinder-agar plates.

#### 1.1. Test organisms

Use the test organism specified in the individual monograph.

#### 1.2. Culture media

Unless otherwise specified, use media with the following compositions. When 'peptone' is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use 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. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

##### (1) Agar media for seed and base layer

##### 1) Media for test organism *Bacillus subtilis* ATCC 6633

i. Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

ii. Peptone	5.0 g
Meat extract	3.0 g
Trisodium citrate dihydrate	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

##### 2) Medium for test organism *Saccharomyces cerevisiae* ATCC 9763

Glucose	10.0 g
Peptone	9.4 g
Meat extract	2.4 g
Yeast extract	4.7 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

**3) Media for other organisms**

i. Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

iii. Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. 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 test organism *Saccharomyces cerevisiae* ATCC 9763**

Glucose	15.0 g
Peptone	5.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

**2) Media for other organisms**

i. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

**1.3. Preparation of agar slant or plate media**

Unless otherwise specified, dispense approximately 9 mL of melted agar medium in each test tube (approximately 16 mm in inside diameter), and make them as slant media, or dispense approximately 20 mL of melted agar medium in each Petri dish (approximately 90 mm in inside diameter), and make them as plate media.

**1.4. Preparation of stock suspensions of test spores or organisms**

Unless otherwise specified, prepare stock suspensions of test spore or organism cultures as follows. Check the aspects of the test spores or organisms as occasion demands.

**(i) Preparation of a stock spore suspension of test organism *Bacillus subtilis* ATCC 6633**

Inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the test organisms specified in 2 (2) 2) i. Incubate at 32 to 37°C for 16 to 24 hours. Inoculate the subcultured test organism onto a suitable volume of slant or plate of the agar medium (described above), which was prepared for transferring the test organisms specified in 2 (2) 2) ii. Then incubate at 32 to 37°C for not less than 1 week to prepare spores. Suspend the spores in isotonic sodium chloride solution, heat at 65°C for 30 minutes, and then centrifuge. Wash the spore sediment three times with isotonic sodium chloride solution by means of centrifugation. Re-suspend the spore sediment in water or isotonic sodium chloride solution, and heat again at 65°C for 30 minutes to prepare the stock spore suspension. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock spore suspension at a temperature not exceeding 5°C, and use within 6 months. If the stock spore suspension shows a clear and definite zone of growth inhibition in an antibiotics potency test using adequate antibiotics, it may be used for further 6 months.

**(ii) Preparation of a stock suspension of the test organism *Saccharomyces cerevisiae* ATCC 9763**

Inoculate test organism onto the slant or plate agar medium which has been prepared for transferring test organism specified in 2 (2) 1). Incubate at 25 to 26°C for 40 to 48 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate at 25 to 26°C for 40 to 48 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 30 days.

**(iii) Preparation of a stock suspension of other test organisms**

Inoculate the test organism onto the slant or the plate of the agar medium which has been prepared for transferring the test organisms specified in 2 (2) 2) i. Incubate the inoculated slant at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organ-

ism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 5 days.

#### 1.5. Preparation of agar base layer plates

Unless otherwise specified, dispense 20 mL of the melted agar medium for the base layer into each Petri dish, and in the case of a large dish, dispense a quantity of the agar medium to form a uniform layer 2 to 3 mm thick. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden.

#### 1.6. Preparation of seeded agar layers

Unless otherwise specified, determine the volume of the stock suspension of the spore or the test organism with which the employed standard solution shows a clear and definite zone of growth inhibition. Prepare the seeded agar layer by mixing thoroughly the previously determined volume of stock suspension of spore or test organism with agar medium for the seed layer kept at 48 to 51°C. Usually, the rate of a stock spore suspension and a stock suspension of the test organism to add to the agar medium for the seed layer are 0.1 to 1.0 vol% and 0.5 to 2.0 vol%, respectively.

#### 1.7. Preparation of cylinder-agar plates

Dispense 4 to 6 mL of the seeded agar layer, which is specified in the individual monograph, on an agar base layer plate in a Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. 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 to 28 mm radius). When large dish plates are used, place cylinders on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cylinders on each large dish plate is considered to be equivalent to one Petri dish plate. Use stainless steel cylinders with the following dimensions: outside diameter 7.9 to 8.1 mm; inside diameter 5.9 to 6.1 mm; length 9.9 to 10.1 mm. The cylinders should not interfere with the test. Prepare the cylinder-agar plates before use.

#### 1.8. Standard solutions

Use both a standard solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

#### 1.9. Sample solutions

Use both a sample solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

#### 1.10. Procedure

Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cylinders for one assay set should be equal to that defined when using Petri dishes. Apply the standard solution of high concentration and that of low concentration to a pair of cylinders set opposite each other on each plate. Apply the high and low concentration sample solutions to the remaining 2 cylinders. The same volume of these solutions must be added to each cylinder.

Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

#### 1.11. 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.

$$d = \alpha \log P + \beta$$

where,  $\alpha$  and  $\beta$  are constants.

If necessary, ascertain the values in the above equation.

Based on this equation, estimate the potency of the sample solutions by application of the following equation:

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

where:

$$\log A = \frac{IV}{W}$$

$$I = \log (\text{potency of } S_H / \text{potency of } S_L)$$

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

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

The sum of the diameter (mm) of the inhibitory zone measured in each plate is designated as follows:

$$\text{for standard solution of high concentration (} S_H) = \sum S_H$$

$$\text{for standard solution of low concentration (} S_L) = \sum S_L$$

$$\text{for sample solution of high concentration (} U_H) = \sum U_H$$

$$\text{for sample solution of low concentration (} U_L) = \sum U_L$$

## 2. Perforated plate method

The perforated plate method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the size of the zone of growth inhibition of a test organism by the use of perforated agar plates.

This method is carried out by the use of perforated agar plates in lieu of cylinder-agar plates used in Cylinder-plate method.

Proceed as directed below, but comply with the requirements of Cylinder-plate method, such as test organisms, media, preparation of agar slant or plate media, preparation of stock suspensions of spores or test organisms, preparation of agar base layer plates, preparation of seeded agar layers, standard solutions, sample solutions, and estimation of potency.

#### 2.1. Preparation of perforated agar plates

Dispense 4 to 6 mL of the seeded agar layer specified in the individual monograph on each agar base layer plate of the Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Using a suitable tool, prepare 4 circular cavities having a diameter of 7.9 to 8.1 mm on a Petri dish agar plate so that the individual cavities are equidistant from the center of the plate. The cavities spaced equally from one another on the circumference of a circle with radius 25 to 28 mm, and are deep enough to reach the bottom of dish. When large dish plates are used, prepare the circular cavities on each plate accord-

ing to the method of preparation for Petri dish agar plates. A set of 4 cavities on each large dish plate is considered to be equivalent to one Petri dish plate. Prepare the perforated agar plates before use.

### 2.2. Procedure

Unless otherwise specified, use 5 perforated agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cavities for one assay set should be equal to that defined when using Petri dishes. Apply the high and low concentration standard solutions to a pair of cavities prepared opposite each other on each plate, and apply the high and low concentration sample solutions to the remaining 2 cavities. The same volume of these solutions must be added to each cavity. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of the circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

### 3. Turbidimetric method

The turbidimetric method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the inhibition of growth of a microbial culture in a fluid medium. The inhibition of growth of a test organism is photometrically measured as changes in turbidity of the microbial culture.

#### 3.1. Test organisms

Use the test organism specified in the individual monograph.

#### 3.2. Culture media

Unless otherwise specified, use media with the following compositions. When peptone is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

##### (1) Agar media for transferring test organisms

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. 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

Glucose	1.0 g
Peptone	5.0 g
Meat extract	1.5 g
Yeast extract	1.5 g
Sodium chloride	3.5 g
Potassium dihydrogen phosphate	1.32 g
Disodium hydrogen phosphate*	3.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization.

\*Dipotassium hydrogen phosphate (3.68 g) may be used in lieu of disodium hydrogen phosphate (3.0 g).

#### 3.3. Preparation of agar slant or plate media

Unless otherwise specified, proceed as directed in Preparation of agar slant or plate media under Cylinder-plate method.

#### 3.4. Preparation of stock suspensions of test organisms

Unless otherwise specified, inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the specified test organism. Incubate the inoculated medium at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Check the aspects of the test spores or organisms as occasion demands. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. After incubation, suspend the test organism in the liquid medium for suspending the test organism, and use as the suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands.

#### 3.5. Standard solutions

Use the standard solutions specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

#### 3.6. Sample solutions

Use the sample solutions specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

#### 3.7. Procedure

Unless otherwise specified, proceed as follows:

Distribute 1.0 mL of each concentration of the standard solution, the sample solution, and water used as a control, into each set composed of 3 test tubes (about 14 mm in inside diameter and about 13 cm in length). Add 9.0 mL of the suspension of the test organism to each tube, and then incubate in a water bath maintained at 35 to 37°C for 3 to 4 hours. After incubation, add 0.5 mL of dilute formaldehyde (1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

#### 3.8. Estimation of potency

Average the transmittance or absorbance values of each concentration of the standard solution, the sample 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 sample 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 equations. 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.

$a, b, c, d, e$  = 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 the values from each geometrically increased concentration standard solution, respectively, and  $e$  is the value from the highest concentration standard solution.

## 4.03 Digestion Test

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

### 1. 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.

#### 1.1. 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.

##### 1.1.1. Preparation of Sample 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 sample 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.

##### 1.1.2. Preparation of Substrate Solution

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

##### 1.1.3. Procedure

Pipet 10 mL of the substrate solution, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes, add exactly 2 mL of alkaline tartrate solution of the Fehling's TS for amylolytic activity test, and shake immediately. Then, add exactly 2 mL of copper solution of the Fehling's TS for amylolytic activity test, shake gently, heat the solution in a water bath for exactly 15 minutes, and then immediately cool to below  $25^\circ\text{C}$ . Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> 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 ( $a$  mL). Separately, pipet 10 mL of water instead of the substrate solution and titrate <2.50> in the same manner ( $b$  mL).

Starch saccharifying activity (unit/g)

$$= \text{amount (mg) of glucose} \times \frac{1}{10} \times \frac{1}{M}$$

Amount (mg) of glucose =  $(b - a) \times 1.6$

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

### 1.2. 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 (amylose) 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.

#### 1.2.1. Preparation of Sample 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 sample 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.

#### 1.2.2. Preparation of Substrate Solution

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

#### 1.2.3. Procedure

Pipet 10 mL of the substrate solution, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes. Pipet 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance  $A_T$  of this solution at the wavelength of 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Separately, using 1 mL of water instead of the sample solution, determine the absorbance  $A_B$  in the same manner.

Starch dextrinizing activity (unit/g)

$$= \frac{(A_B - A_T)}{A_B} \times \frac{1}{M}$$

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

### 1.3. 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.

#### 1.3.1. Preparation of Sample 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 sample 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.

### 1.3.2. 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 300 mL of water, then add gradually 25 mL 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 it occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

### 1.3.3. Preparation of 50% Standard Sucrose Solution

Dissolve 50.0 g of sucrose in 50.0 mL of water.

### 1.3.4. Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL conical flask, and allow it to stand in a thermostat at  $37 \pm 0.5^\circ\text{C}$  for 15 minutes. Fix a viscometer shown in Fig. 4.03-1 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 pulling up 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 100-mL conical flask, and stand it in another thermostat at  $37 \pm 0.5^\circ\text{C}$  for 20 minutes. Add exactly 1 mL of the sample 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 pull the reac-

tion solution up 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 sample 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 correspond 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 sample in 1 mL of sample solution

## 2. Assay for Protein Digestive Activity

The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin's reaction, of the amount of acid-soluble low-molecular 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's TS-colorable substance equivalent to  $1 \mu\text{g}$  of tyrosine per minute under the conditions described in Procedure.

### 2.1. Preparation of Sample 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's TS-colorable substances increase in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 30 protein digestive activity unit/mL.

### 2.2. 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 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make them exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each solution, shake immediately, then stand them at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes. Determine the absorbances,  $A_1$ ,  $A_2$ ,  $A_3$  and  $A_4$ , of these solutions at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with exactly 2 mL 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 ( $\mu\text{g}$ ) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount ( $\mu\text{g}$ ) of tyrosine for the absorbance 1.

### 2.3. Preparation of Substrate Solution

(i) Substrate solution 1: 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 12 mL of lactic acid TS and 150 mL 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 200 mL. Prepare before use.

(ii) Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2

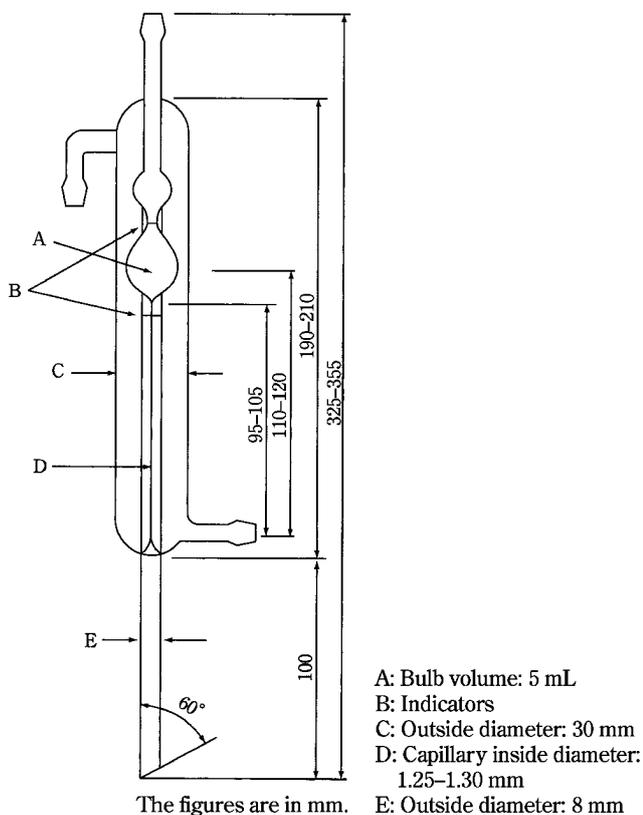


Fig. 4.03-1

hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160 mL of 0.05 mol/L disodium 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 200 mL. Prepare before use.

#### 2.4. Preparation of Precipitation Reagent

(i) Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.

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

#### 2.5. Procedure

Pipet 5 mL of the substrate solution specified in the monograph, stand at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. After standing this solution at  $37 \pm 0.5^\circ\text{C}$  for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes, and then filter. Discard the first 3 mL of the filtrate, exactly measure the subsequent 2 mL of the filtrate, add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to the solution, shake well, and stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes. Determine the absorbance  $A_T$  of this solution at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at  $37 \pm 0.5^\circ\text{C}$  for 30 minutes. Follow the same procedure for the sample 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 sample in 1 mL of sample solution

*F*: Amount ( $\mu\text{g}$ ) 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  $\mu\text{mole}$  of fatty acid per minute under the conditions described in Procedure.

#### 3.1. Preparation of Sample 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 sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

#### 3.2. Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender (see Fig. 4.03-2), and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below  $10^\circ\text{C}$ .

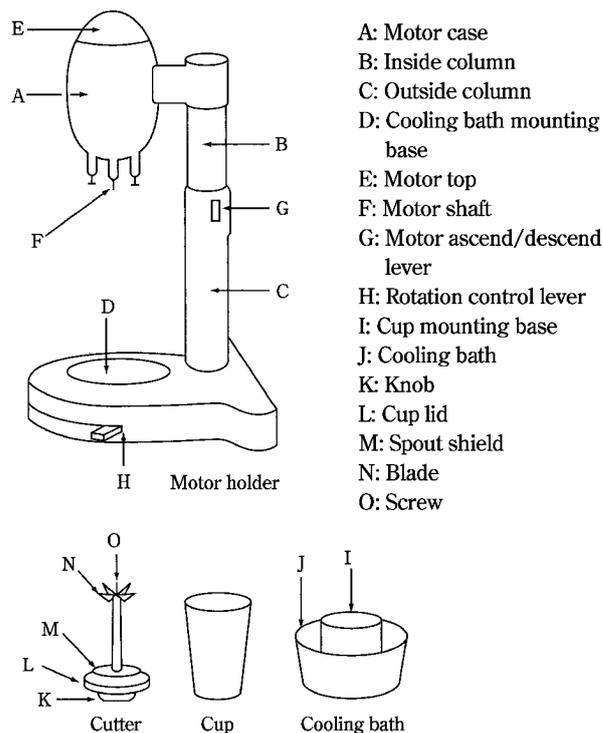


Fig. 4.03-2 Blender

Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

#### 3.3. Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between  $75^\circ\text{C}$  and  $80^\circ\text{C}$  for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

#### 3.4. Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at  $37 \pm 0.5^\circ\text{C}$  for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate <2.50> the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (*b* mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at  $37 \pm 0.5^\circ\text{C}$  for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate <2.50> 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 sample in 1 mL of sample solution

## 4.04 Pyrogen Test

Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

### 1. 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.

### 2. Apparatus, instruments

(i) Thermometer—Use a rectal thermometer or temperature-measuring apparatus with an accuracy of  $\pm 0.1^\circ\text{C}$  or less.

(ii) Syringe and injection needle—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.

### 3. Test procedures

#### 3.1. Quantity of injection

Unless otherwise specified, inject 10 mL of the sample per kg of body mass of each rabbit.

#### 3.2. 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. The “control temperature” of each rabbit is the mean of two temperature readings recorded for that rabbit at an interval of 30 min in the 40 min immediately preceding the injection of the sample to be examined. Rabbits showing a temperature variation greater than 0.2°C between the two successive temperature readings or rabbits having an initial temperature higher than 39.8°C are withdrawn from the test.

Warm the test solution to a temperature of  $37 \pm 2^\circ\text{C}$  before injection, and inject the solution slowly into the marginal vein of the ear of each rabbit over a period not exceeding 10 min. 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 maximum temperature of each rabbit is taken to be the rise in body temperature. Consider any temperature decreases as zero rise.

### 4. Interpretation of results

The test is carried out on a group of three rabbits and the result is judged on the basis of the sum of the three temperature rises. Repeat if necessary on further groups of three rabbits to a total of three groups, depending on the results obtained. If the summed response of the first group does not exceed 1.3°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 2.5°C, the sample is judged to be pyrogen-positive. If the summed response exceed 1.3°C but does not exceed 2.5°C, repeat the test on another group of three rabbits. If the summed response of the first and second group does not exceed 3.0°C, the sample is judged to be pyrogen-negative. If the summed response of the 6 rabbits exceeds 4.2°C, the sample is judged to be pyrogen-positive. If the summed response exceeds 3.0°C but does not exceed 4.2°C, repeat the test on one more group of three rabbits. If the summed response of the 9 rabbits does not exceed 5.0°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 5.0°C, the sample is judged to be pyrogen-positive.

When the test sample is judged to be pyrogen-negative, the sample passes the pyrogen test.

## 4.05 Microbial Limit Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

This chapter includes microbial enumeration tests and tests for specified micro-organisms. 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 test specimens 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

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may 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 micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

#### 1. 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 micro-organisms 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 micro-organisms must be demonstrated.

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

## 2. Enumeration 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 micro-organisms. 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.

## 3. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

The ability of the test to detect micro-organisms 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.

### 3.1. 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 micro-organisms 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 4.05-I-1.

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 per cent of polysorbate 80 may be added to the buffer. Use the suspensions within 2 h or within 24 h 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.

### 3.2. Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 4. Testing of Products. A failed negative control requires an investigation.

### 3.3. Growth promotion of the 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 micro-organisms indicated in Table 4.05-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 4.05-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 4.05-I-1.

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

## 3.4. Suitability of the counting method in the presence of product

### 3.4.1. 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 a 1 in 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 to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

(ii) Non-fatty products insoluble in water: Suspend the product to be examined (usually a 1 in 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 wettable substances. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

(iii) Fatty products: Dissolve in isopropyl myristate, sterilised 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, or 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 pre-warmed chosen diluent to make a 1 in 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 tenfold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

(iv) Fluids or solids in aerosol form: Aseptically transfer the product into 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 ("release liner") 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 diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least

Table 4.05-I-1 Preparation and use of test micro-organisms

Micro-organism	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
<i>Staphylococcus aureus</i>  such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	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	
<i>Pseudomonas aeruginosa</i>  such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	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	
<i>Bacillus subtilis</i>  such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134	Soybean-Casein Digest Agar Medium or Fluid Soybean-Casein Digest Medium 30 – 35°C 18 – 24 h	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	
<i>Candida albicans</i>  such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594	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
<i>Aspergillus brasiliensis</i>  such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455	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

30 min.

#### 3.4.2. Inoculation and dilution

Add to the sample prepared as described above (3.4.1.) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution 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.

#### 3.4.3. Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 3.4.2. and incubated following the procedure described in 3.4.4., is compared to

Table 4.05-I-2 Common neutralizing agents/method for interfering substances

Interfering substance	Potential neutralizing agents/method
Glutaraldehyde, Mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
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

the number of micro-organisms 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 diluent, (3) membrane filtration or (4) a combination of the above measures.

**Neutralizing agents**—Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 4.05-I-2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizing agents, without product.

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 micro-organism. However, it is possible that the product only inhibits some of the micro-organisms 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.

#### 3.4.4. Recovery of micro-organism in the presence of product

For each of the micro-organisms listed in Table 4.05-I-1, separate tests are performed. Only micro-organisms of the added test strain are counted.

##### 3.4.4.1. Membrane filtration method

Use membrane filters having a nominal pore size not greater than 0.45  $\mu\text{m}$ . The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed in Table 4.05-I-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under 3.4.1. to 3.4.3. (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 4.05-I-1. Perform the counting.

##### 3.4.4.2. Plate-count methods

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

(i) Pour-plate method: For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 3.4.1. to 3.4.3. and 15 – 20 mL 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 of agar medium is increased accordingly. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 4.05-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(ii) Surface-spread method: For Petri dishes 9 cm in diameter, add 15 – 20 mL 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 cabinet or in an incubator. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under 3.4.1. to 3.4.3. over the surface of the medium. Incubate and count as prescribed under 3.4.4.2. (i).

##### 3.4.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the 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.

Table 4.05-I-3 Most-probable-number values of micro-organisms

Observed combinations of numbers of tubes showing growth in each set			MPN per g or per mL of product	95 per cent confidence limits
Number of g or mL 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	

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 3.4.1. to 3.4.3. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3

tubes with 9 – 10 mL of Fluid Soybean-Casein Digest Medium. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added

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 micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

### 3.5. 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 3.4.2. in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum 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.

## 4. Testing of Products

### 4.1. Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL 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 10 mL 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 1000 mL or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorised.

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.

### 4.2. Examination of the product

#### 4.2.1. 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 3 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 the number of CFU per gram or per millilitre of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under 3.4.1. 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.

#### 4.2.2. 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 millilitre 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.

#### 4.2.3. Most-probable-number method

Prepare and dilute the sample using a method that has been shown to be suitable as described in section 1.3. 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 micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

### 4.3. 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:

- 10<sup>1</sup> CFU: maximum acceptable count = 20,
- 10<sup>2</sup> CFU: maximum acceptable count = 200,
- 10<sup>3</sup> CFU: maximum acceptable count = 2000, and so forth.

The recommended solutions and media are described in *Tests for specified micro-organisms*.

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

These tests are harmonized with the European Phar-

macopoeia and the U.S. Pharmacopoeia.

The tests described hereafter will allow determination of the absence of, or limited occurrence of specified micro-organisms 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 samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including automated methods may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

### 1. 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 micro-organisms and their compatibility with inactivators used must be demonstrated as described in I. Total viable aerobic count.

### 2. Growth Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls

The ability of the test to detect micro-organisms 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.

#### 2.1. 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 micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

##### 2.1.1. Aerobic micro-organisms

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 or NBRC 13276,

*Pseudomonas aeruginosa* such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

*Escherichia coli* such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

*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 or NBRC 1594.

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.

#### 2.1.2. 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 anaerobic 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*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period.

#### 2.2. Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 3. A failed negative control required an investigation.

#### 2.3. 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 4.05-II-1.

(i) 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 micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(ii) 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 micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(iii) Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

(iv) Test for indicative properties: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. 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.

#### 2.4. Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 3. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 3 using the shortest incubation period prescribed.

Table 4.05-II-1 Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
<b>Test for bile-tolerant gram-negative bacteria</b>		
Fluid Enterobacteria Enrichment Broth Mossel Medium	Growth promoting	<i>E. coli</i> <i>P. aeruginosa</i>
	Inhibitory	<i>S. aureus</i>
VRB (Violet/Red/Bile) Agar with glucose	Growth promoting + Indicative	<i>E. coli</i> <i>P. aeruginosa</i>
<b>Test for <i>Escherichia coli</i></b>		
Fluid MacConkey Broth Medium	Growth promoting	<i>E. coli</i>
	Inhibitory	<i>S. aureus</i>
MacConkey Agar Medium	Growth promoting + Indicative	<i>E. coli</i>
<b>Test for <i>Salmonella</i></b>		
Fluid Rappaport Vassiliadis Salmonella Enrichment Broth Medium	Growth promoting	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Inhibitory	<i>S. aureus</i>
XLD (Xylose-Lysine-Desoxycholate) Agar Medium	Growth promoting + Indicative	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Indicative	<i>E. coli</i>
<b>Test for <i>Pseudomonas aeruginosa</i></b>		
Cetrimide Agar Medium	Growth promoting	<i>P. aeruginosa</i>
	Inhibitory	<i>E. coli</i>
<b>Test for <i>Staphylococcus aureus</i></b>		
Mannitol Salt Agar Medium	Growth promoting + Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
<b>Test for Clostridia</b>		
Reinforced Clostridial Medium	Growth promoting	<i>Cl. sporogenes</i>
Columbia Agar Medium	Growth promoting	<i>Cl. sporogenes</i>
<b>Test for <i>Candida albicans</i></b>		
Fluid Sabouraud Glucose Medium	Growth promoting	<i>C. albicans</i>
Sabouraud Glucose Agar Medium	Growth promoting + Indicative	<i>C. albicans</i>

The specified micro-organisms must be detected with the indication reactions as described in section 3.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see I. of Total viable aerobic count).

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralised, then it is to be assumed that the inhibited micro-organism will not be present in the product.

### 3. Testing of Products

#### 3.1. Bile-tolerant gram-negative bacteria

##### 3.1.1. 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 diluent, mix and incubate at 20 – 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

##### 3.1.2. 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.

##### 3.1.3. Quantitative test

###### 3.1.3.1. Selection and subculture

Inoculate suitable quantities of Fluid Enterobacteria Enrichment Broth Mossel Medium with the preparation as described under 3.1.1. and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) 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.

###### 3.1.3.2. Interpretation

Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 4.05-II-2 the probable number of bacteria.

#### 3.2. *Escherichia coli*

##### 3.2.1. 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 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

##### 3.2.2. Selection and subculture

Shake the container, transfer 1 mL of Fluid Soybean-Casein Digest Medium to 100 mL of Fluid MacConkey Broth Medium 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.

##### 3.2.3. 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.

#### 3.3. *Salmonella*

##### 3.3.1. 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 10 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium, mix and incubate at 30 – 35°C for 18 – 24 hours.

##### 3.3.2. Selection and subculture

Transfer 0.1 mL of Fluid Soybean-Casein Digest Medium to 10 mL 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.

##### 3.3.3. Interpretation

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres. 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.

#### 3.4. *Pseudomonas aeruginosa*

##### 3.4.1. 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 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) 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 (3.4.1.) through a sterile filter membrane and place in 100 mL of Fluid Soybean-Casein Digest Medium. Incubate at 30 – 35°C for 18 – 24 hours.

##### 3.4.2. Selection and subculture

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

##### 3.4.3. Interpretation

Growth 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.

Table 4.05-II-2 Interpretation of results

Results for each quantity of product			Probable number of bacteria per gram or mL of product
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	more than 10 <sup>3</sup>
+	+	–	less than 10 <sup>3</sup> and more than 10 <sup>2</sup>
+	–	–	less than 10 <sup>2</sup> and more than 10
–	–	–	less than 10

**3.5. Staphylococcus aureus****3.5.1. 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 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of Fluid Soybean-Casein Digest Medium and homogenise. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. Total viable aerobic count (3.4.1.) through a sterile filter membrane and place in 100 mL of Fluid Soybean-Casein Digest Medium. Incubate at 30 – 35°C for 18 – 24 hours.

**3.5.2. Selection and subculture**

Subculture on a plate of Mannitol Salt Agar Medium and incubate at 30 – 35°C for 18 – 72 hours.

**3.5.3. 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.

**3.6. Clostridia****3.6.1. 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.

**3.6.2. Selection and subculture**

Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under 2.4.) 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.

**3.6.3. 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.

**3.7. Candida albicans****3.7.1. Sample preparation and pre-incubation**

Prepare the product to be examined as described in I. Microbial Enumeration Tests and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL to inoculate 100 mL of Fluid Sabouraud Glucose Medium and mix. Incubate at 30 – 35°C for 3-5 days.

**3.7.2. Selection and subculture**

Subculture on a plate of Sabouraud Glucose Agar Medium and incubate at 30 – 35°C for 24 – 48 hours.

**3.7.3. 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 nega-

tive.

The following section is given for information.

**4. Recommended Solutions and Culture Media**

The following solutions and culture media have been found satisfactory for the purposes 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.

**(i) Phosphate Buffer (pH 7.2)**

Prepare a mixture of water and stock buffer solution (800:1 V/V) and sterilize.

*Stock buffer solution.* Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500 mL 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.

**(ii) Buffered Sodium Chloride-Peptone Solution (pH 7.0)**

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g
(equivalent to 0.067 mol phosphate)	
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave using a validated cycle.

**(iii) 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.

**(iv) 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.

**(v) 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.

**(vi) 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.

(vii) Fluid Sabouraud Glucose Medium	
Glucose	20.0 g
Peptones (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.

(viii) 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 heating it is 7.0 – 7.4 at 25°C. Heat at 100°C for 30 min and cool immediately.

(ix) 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 heating it is 7.2 – 7.6 at 25°C. Heat to boiling; do not heat in an autoclave.

(x) 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	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.

(xi) MacConkey Agar Medium	
Gelatin peptone	17.0 g
Peptones (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.

(xii) 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
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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.

(xiii) 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.

(xiv) Cetrinide Agar Medium	
Gelatin peptone	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulfate	10.0 g
Cetrinide	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.

(xv) 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.

(xvi) 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.

## (xvii) 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.

## 4.06 Sterility Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

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 micro-organism has been found in the sample examined in the conditions of the test.

### 1. 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 micro-organisms 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.

### 2. Culture media and incubation temperatures

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.

#### (i) Fluid thioglycollate medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or Thioglycollic acid	0.5 g 0.3 mL
Resazurin sodium solution (1 in 1000), freshly prepared	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.

#### (ii) 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 monohydrate/anhydrous	2.5/2.3 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.

### 3. Suitability of the culture medium

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

#### 3.1. Sterility

Incubate portions of the media for 14 days. No growth of micro-organisms occurs.

**Table 4.06-1.** Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Method suitability Test

Aerobic bacteria <i>Staphylococcus aureus</i>	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
<i>Bacillus subtilis</i>	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
<i>Pseudomonas aeruginosa</i>	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium <i>Clostridium sporogenes</i>	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi <i>Candida albicans</i>	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
<i>Aspergillus brasiliensis</i>	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

**3.2. 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 4.06-1.

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 micro-organism:

*Clostridium sporogenes*,  
*Pseudomonas aeruginosa*,  
*Staphylococcus aureus*.

Inoculate portions 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 micro-organism:

*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 micro-organisms occurs.

**4. Method suitability test**

Carry out a test as described below under 5. Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

(i) 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 micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

(ii) Direct inoculation: After transferring the contents of the container or containers to be tested to the culture medi-

um add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms 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 5 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 conditions 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:

- when the test for sterility has to be carried out on a new product;
- 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.

**5. Test for sterility of the product to be examined**

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.

**5.1. Membrane filtration**

Use membrane filters having a nominal pore size not greater than 0.45  $\mu\text{m}$  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 diluent such as a 1 g/L neutral solution of meat or casein peptone pH 7.1  $\pm$  0.2 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be

**Table 4.06-2.** Minimum quantity to be used for each medium

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorized
Liquids	
—less than 1 mL:	The whole contents of each container
—1 – 40 mL:	Half the contents of each container but not less than 1 mL
—greater than 40 mL and not greater than 100 mL	20 mL
—greater than 100 mL:	10% of the contents of the container but not less than 20 mL
Antibiotic liquids	1 mL
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
—less than 50 mg	The whole contents of each container
—50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
—300 mg – 5 g	150 mg
—greater than 5 g	500 mg

tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 4.06-2. 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 diluent 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.

(ii) Soluble solids: Use for each medium not less than the quantity prescribed in Table 4.06-2 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 4.06-2. 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 diluent 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 4.06-2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent 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.

### 5.2. Direct inoculation of the culture medium

Transfer the quantity of the preparation to be examined prescribed in Table 4.06-2 directly into the culture medium so that the volume of the product is not more than 10 per cent 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 diluent 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 micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

### 6. 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 1 mL) 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 exa-

**Table 4.06-3.** Minimum number of items to be tested

Number 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 containers	10% or 4 containers whichever is the greater 10 containers  2% or 20 containers ♦(10 containers for parenterals with a nominal volume of 100 mL or more)♦, whichever is 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 whichever is 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 whichever is the greater  2% or 10 containers whichever is the greater

\* If the batch size is not known, use the maximum number of items prescribed

\*\* If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

mined 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:

(i) the data of the microbiological monitoring of the sterility testing facility show a fault;

(ii) a review of the testing procedure used during the test in question reveals a fault;

(iii) microbial growth is found in the negative controls;

(iv) 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.

### 7. 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 4.06-2, 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 4.06-2, unless otherwise justified and authorized. The tests 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 tests, the contents of two or more containers are used to inoculate the different media.

### 8. 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 4.06-3.

## 5. Tests for Crude Drugs

### 5.01 Crude Drugs Test

Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

#### 1. Sampling

Unless otherwise specified, sample should be taken by the following methods. If necessary, preserve the samples in tight containers.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.

(iii) When the mass of each single piece of the crude drugs is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly.

#### 2. Preparation of the test sample for analysis

Preparations are to be made by mixing the sample well. Powdered drugs should be used as they are, and in the case of unpowdered drugs, unless otherwise specified, grind the sample into powder. If the sample cannot be ground into powder, reduce it as finely as possible, spread it out in a thin layer, and withdraw a typical portion for analysis. If necessary, preserve the test sample in a tight container.

#### 3. Microscopic examination

##### 3.1. Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

##### 3.2. Preparation for microscopic examination

(i) Section: To a section on a slide glass add 1 to 2 drops

of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20  $\mu\text{m}$  in thickness.

(ii) Powder: Place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for a while to swell the sample. Add 1 drop of the mounting agent, and put a cover glass on it so that the tissue sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles. In the case where the tissue sections are opaque, place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of chloral hydrate TS, heat to make the tissues clear while stirring with a small glass rod to prevent boiling. After cooling, add 1 drop of mounting agent, and put a cover glass on it in the same manner as above.

Unless otherwise specified, use a mixture of glycerin and water (1:1) or a mixture of water, ethanol (95) and glycerin (1:1:1) as the mounting agent and swelling agent.

### 3.3. Observation of components in the Description

In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

## 4. Purity

### 4.1. Foreign matter

Unless otherwise specified, weigh 25 to 500 g of the sample, spread out in a thin layer, and separate the foreign matter by inspecting with the naked eye or with the use of a magnifying glass of 10 magnifications. Weigh, and determine the percentage of foreign matter.

### 4.2. Total BHC's and total DDT's

Sodium chloride, anhydrous sodium sulfate and synthetic magnesium silicate for column chromatography used in this procedure are used after drying by heating at about 130°C for more than 12 hours and cooling in a desiccator (silica gel). Chromatographic column is prepared as follows: Place 20 g of synthetic magnesium silicate for column chromatography in a 200-mL flask, add 50 mL of hexane for Purity of crude drug, shake vigorously, and immediately pour the mixture into a chromatographic tube about 2 cm in inside diameter and about 30 cm in length. Drip until the depth of hexane layer at the upper part is about 5 cm, introduce 8 g of anhydrous sodium sulfate from the top, and further drip until a small quantity of hexane is left at the upper part.

Weigh accurately about 5 g of pulverized sample, place in a glass-stoppered centrifuge tube, add 30 mL of a mixture of acetone for Purity of crude drug and water (5:2), stopper tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with the residue using two 30-mL portions of the mixture of acetone for Purity of crude drug and water (5:2). Combine all the supernatant liquids, and concentrate under reduced pressure at a temperature not higher than 40°C until the order of acetone is faint. Transfer the concentrated solution to a separator containing 100 mL of sodium chloride TS, and shake twice with two 50-mL portions of hexane for Purity of crude drug for 5 minutes each. Combine the hexane layers, trans-

fer to a separator containing 50 mL of sodium chloride TS, and shake for 5 minutes. Take the hexane layer, dry with 30 g of anhydrous sodium sulfate, and filter. Wash the residue on the filter paper with 20 mL of hexane for Purity of crude drug. Combine the filtrate and the washings, and concentrate under reduced pressure at a temperature not higher than 40°C to about 5 mL. Transfer this solution to the chromatographic column and allow to pass with 300 mL of a mixture of hexane for Purity of crude drug and diethyl ether for Purity of crude drug (17:3) at a rate of not more than 5 mL per minute. After concentrating the eluate under reduced pressure at a temperature not higher than 40°C, add hexane for Purity of crude drug to make exactly 5 mL. Transfer this solution to a glass-stoppered test tube, add 1 mL of sulfuric acid, and shake carefully. Take 4 mL of the upper layer, transfer to a separate glass-stoppered test tube, add 2 mL of water, and shake gently. Take 3 mL of the upper layer so obtained, transfer to a glass-stoppered centrifuge tube, dry with 1 g of anhydrous sodium sulfate, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg each of  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE, dissolve in 5 mL of acetone for Purity of crude drug, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 10 mL of this solution, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for Purity of crude drug to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 1  $\mu\text{L}$  each of the sample solution and the standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas corresponding to  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE,  $A_{TA}$  and  $A_{SA}$ ;  $A_{TB}$  and  $A_{SB}$ ;  $A_{TC}$  and  $A_{SC}$ ;  $A_{TD}$  and  $A_{SD}$ ;  $A_{TE}$  and  $A_{SE}$ ;  $A_{TF}$  and  $A_{SF}$ ;  $A_{TG}$  and  $A_{SG}$ ;  $A_{TH}$  and  $A_{SH}$ . Calculate the content of each of  $\alpha$ -BHC,  $\beta$ -BHC,  $\gamma$ -BHC,  $\delta$ -BHC,  $o,p'$ -DDT,  $p,p'$ -DDT,  $p,p'$ -DDD and  $p,p'$ -DDE by means of the following equations.

Content (ppm) of  $\alpha$ -BHC

$$= \frac{\text{amount (g) of } \alpha\text{-BHC}}{M} \times \frac{A_{TA}}{A_{SA}} \times 50$$

Content (ppm) of  $\beta$ -BHC

$$= \frac{\text{amount (g) of } \beta\text{-BHC}}{M} \times \frac{A_{TB}}{A_{SB}} \times 50$$

Content (ppm) of  $\gamma$ -BHC

$$= \frac{\text{amount (g) of } \gamma\text{-BHC}}{M} \times \frac{A_{TC}}{A_{SC}} \times 50$$

Content (ppm) of  $\delta$ -BHC

$$= \frac{\text{amount (g) of } \delta\text{-BHC}}{M} \times \frac{A_{TD}}{A_{SD}} \times 50$$

Content (ppm) of  $o,p'$ -DDT

$$= \frac{\text{amount (g) of } o,p'\text{-DDT}}{M} \times \frac{A_{TE}}{A_{SE}} \times 50$$

Content (ppm) of  $p,p'$ -DDT

$$= \frac{\text{amount (g) of } p,p'\text{-DDT}}{M} \times \frac{A_{TF}}{A_{SF}} \times 50$$

Content (ppm) of *p,p'*-DDD

$$= \frac{\text{amount (g) of } p,p' - \text{DDD}}{M} \times \frac{A_{\text{TG}}}{A_{\text{SG}}} \times 50$$

Content (ppm) of *p,p'*-DDE

$$= \frac{\text{amount (g) of } p,p' - \text{DDE}}{M} \times \frac{A_{\text{TH}}}{A_{\text{SH}}} \times 50$$

*M*: Amount (g) of pulverized sample

Content (ppm) of total BHC's

$$= \text{content (ppm) of } \alpha\text{-BHC} + \text{content (ppm) of } \beta\text{-BHC} + \text{content (ppm) of } \gamma\text{-BHC} + \text{content (ppm) of } \delta\text{-BHC}$$

Content (ppm) of total DDT's

$$= \text{content (ppm) of } o,p'\text{-DDT} + \text{content (ppm) of } p,p'\text{-DDT} + \text{content (ppm) of } p,p'\text{-DDD} + \text{content (ppm) of } p,p'\text{-DDE}$$

*Operating conditions*—

Detector: An electron capture detector.

Sample injection system: A splitless injection system.

Column: A fused silica capillary column about 0.3 mm in inside diameter and about 30 m in length, coated the inside wall with 7% cyanopropyl-7% phenylmethylsilicone polymer for gas chromatography in a thickness of 0.25 to 1.0  $\mu\text{m}$ .

Column temperature: Maintain the temperature at 60°C for 2 minutes after injection, program to increase the temperature at a rate of 10°C per minute to 200°C, and then program to increase the temperature at a rate of 2°C per minute to 260°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention times of the objective compounds are between 10 and 30 minutes.

*System suitability*—

Test for required detectability: To exactly 1 mL of the standard solution add hexane to make exactly 10 mL. Confirm that the peak area of each objective compound obtained with 1  $\mu\text{L}$  of this solution is equivalent to 5 to 15% of that of corresponding compound with 1  $\mu\text{L}$  of the standard solution.

System performance: When the procedure is run with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the peaks of the object compounds separate completely each other.

System repeatability: Repeat the test 6 times with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of each object compound is not more than 10%.

## 5. Loss on drying

Unless otherwise specified, transfer 2 to 6 g of the test sample for analysis to a tared weighing bottle, and weigh accurately. Dry at 105°C for 5 hours, allow to cool in a desiccator (silica gel), and weigh accurately. Continue the drying at 105°C, and weigh accurately at 1-hour intervals. When the mass of the sample becomes constant, the loss of mass represents the percentage of loss on drying (%). When the period of time for drying is specified, weigh accurately after drying for the period of time specified, and determine the loss on drying (%).

## 6. Total ash

Ignite previously a crucible of platinum, quartz or porcelain between 500°C and 550°C for 1 hour. Cool, and weigh accurately the crucible. Unless otherwise specified, weigh accurately 2 to 4 g of the test sample for analysis in this crucible, take off the lid or keep it open a little if necessary, heat the crucible at a low temperature at first, then gradually heat to a temperature between 500°C and 550°C, ignite to incinerate the residue for more than 4 hours until no carbonized substance remains in the ash, cool, and weigh accurately the ash. Incinerate repeatedly to constant mass, cool, weigh accurately, and determine the amount (%) of total ash. If a carbonized substance remains and a constant mass cannot be obtained in the above-mentioned method, extract the charred mass with hot water, collect the insoluble residue on filter paper for assay, and incinerate the residue and filter paper until no carbonized substance remains in the ash. Then add the filtrate, evaporate it to dryness, and incinerate. Cool, weigh accurately, and determine the mass (%) of the total ash. If a carbon-free ash cannot be obtained even in this way, moisten the ash with a small amount of ethanol (95), break up the ash with a glass rod, wash the rod with a small amount of ethanol (95), evaporate carefully, and determine the mass of the total ash as described above. A desiccator (silica gel) is used for cooling.

## 7. Acid-insoluble ash

Add carefully 25 mL of dilute hydrochloric acid to the total ash, boil gently for 5 minutes, collect the insoluble matter on filter paper for assay, and wash thoroughly with hot water. Dry the residue together with the filter paper, and ignite to incinerate in a tared crucible of platinum, quartz or porcelain for 3 hours. Cool in a desiccator (silica gel), weigh, and determine the amount (%) of acid-insoluble ash. When the amount determined exceeds the limit specified, incinerate repeatedly to a constant mass.

## 8. Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

### 8.1. Dilute ethanol-soluble extract

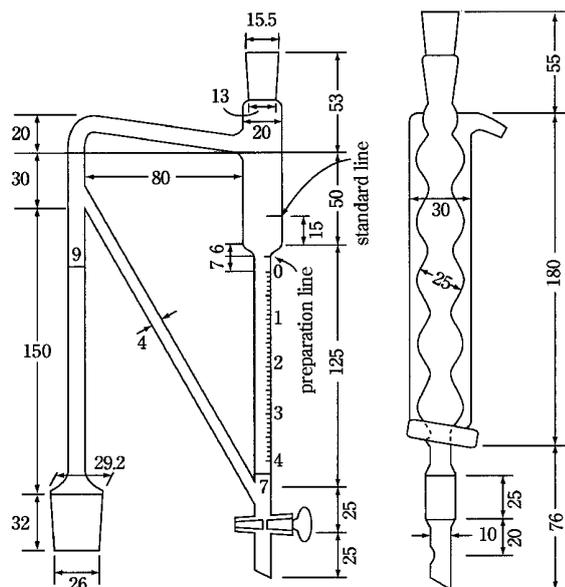
Unless otherwise specified, weigh accurately about 2.3 g of the sample for analysis, extract with 70 mL of dilute ethanol in a suitable flask with occasional shaking for 5 hours, and allow to stand for 16 to 20 hours. Filter, and wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness, dry at 105°C for 4 hours, and cool in a desiccator (silica gel). Weigh accurately the amount, multiply it by 2, and determine the amount of dilute ethanol-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

### 8.2. Water-soluble extract

Proceed as directed in 8.1., using water instead of dilute ethanol, weigh accurately the amount, multiply by 2, and determine the amount of water-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

### 8.3. Diethyl ether-soluble extract

Unless otherwise specified, dry the test sample for analysis in a desiccator (silica gel) for 48 hours, weigh accurately about 2 g of it, and place in a suitable flask. Add 70 mL of diethyl ether, attach a reflux condenser to the flask, and boil



The figures are in mm.

Fig. 5.01-1

Fig. 5.01-2

gently on a water bath for 4 hours. Cool, filter, and wash the flask and the residue with small portions of diethyl ether until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness on a water bath, dry in a desiccator (silica gel) for 24 hours, weigh accurately the amount, multiply it by 2, determine the amount of diethyl ether-soluble extract, and calculate the extract content (%).

## 9. Essential oil content

The test of essential oil content in crude drugs is performed as directed in the following method.

### 9.1. Essential oil determination

Weigh the quantity of the test sample for analysis directed in the monograph in a 1-L hard glass-stoppered flask, and add from 5 to 10 times as much water as the drug. Set up an apparatus for essential oil determination (Fig. 5.01-1), inserting a reflux condenser (Fig. 5.01-2) in the upper mouth of it, and heat the content of the flask in an oil bath between 130°C and 150°C to boiling. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2.0 mL of xylene is added to the graduated tube. Unless otherwise specified, continue boiling for 5 hours, allow to stand for some time, and open the stopper of the apparatus. Draw off the water slowly until the surface of the oil layer corresponds to the preparation line, and allow it to stand for more than 1 hour at ordinary temperature. Then lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil at ordinary temperature. Subtract the volume (mL) of xylene from the volume of the total oil.

## 5.02 Microbial Limit Test for Crude Drugs

This chapter provides tests for the qualitative and quantitative estimation of viable microorganisms present in crude drugs. It includes tests for total viable count (aerobic bacteria and fungi) and specified microbial species (*Enterobacteria* and other gram-negative bacteria, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*). Microbial limit test must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test. When test specimens have antimicrobial activity, or contain antimicrobial substances, any such antimicrobial properties must be eliminated by means of procedures such as dilution, filtration, neutralization or inactivation. 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 test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

### 1. Total viable aerobic count

This test determines mesophilic aerobic bacteria and fungi (molds and yeasts) which grow under aerobic conditions. Psychrophilic, thermophilic, basophilic, and anaerobic bacteria, and microorganisms which require specific ingredients for growth, may give a negative result, even if a significant number exists in the test specimens. The test may be carried out using one of the following 4 methods, i.e., pour plate method, spread plate method, serial dilution method (most probable number method) or membrane filtration method. Use an appropriate method depending on the purpose. An automated method may be used for the test presented here, provided it has been properly validated as giving equivalent or better results. Different culture media and temperature are required for the growth of bacteria and fungi (molds and yeasts). The serial dilution method is applicable only to the enumeration of bacteria.

#### 1.1. Sampling and Preparation of the test specimens

Unless otherwise specified, samples should be taken by the following methods.

(i) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(ii) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly and cutting.

(iii) When the mass of each single piece of the crude drug is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly. If necessary, cut more for use.

(iv) When crude drugs to be sampled are in the form of a solution or a preparation, the sample should be taken after mixing thoroughly.

(v) An insoluble solid should be taken after reducing the substance to a moderately fine powder.

#### 1.2. Preparation of the test fluid

Phosphate Buffer, pH 7.2, Buffered Sodium Chloride-Peptone Solution, pH 7.0 or fluid medium used for the test is used to suspend or dilute the test specimen. Unless other-

wise specified, usually take 10 g or 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A test specimen as a suspension must be shaken for 10 minutes. If necessary, for crude drugs to which microorganisms might adhere, repeat the same method and use this as the test fluid. A different quantity or volume may be used if the nature of the test specimen requires it. The pH of the test fluid is adjusted to between 6 and 8. The test fluid must be used within an hour after preparation.

(i) Fluid specimen: Take 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A different quantity or volume may be used if the nature of the test specimen requires it.

(ii) Insoluble solids: Pulverize the substance to a moderately fine powder, take 10 g of the test specimen, and suspend it in 90 mL of the buffer or fluid medium specified. A different quantity or a larger volume of buffer and fluid medium than indicated may be used for the suspension, if the nature of the test specimen requires it. The suspension may be dispersed well using, if necessary, a mechanical blender. A suitable surface active agent (such as 0.1 w/v% Polysorbate 80) may be added to aid dissolution.

### 1.3. Test procedures

#### 1.3.1. Pour Plate Method

Use petri dishes 9 to 10 cm in diameter. Use at least two petri dishes for each dilution. Pipet 1 mL of the test fluid or its diluted solution onto each petri dish aseptically. Promptly add to each dish 15 to 20 mL of sterilized agar medium that has previously been melted and kept below 45°C, and mix. Primarily for the detection of aerobic microbes, use Casein Soya Bean Digest Agar Medium. For specimens that consist of fragments of crude drugs, or to control the growth of fungi, TTC TS for aerobic bacterial strains and amphotericin B TS as an antimycotic may be added to the agar. Just prior to use, add 2.5–5 mL of TTC TS or 2 mL of amphotericin B TS per liter of sterile medium and mix. Primarily for the detection of fungi, use one of Sabouraud-Dextrose Agar Medium with Antibiotics, Potato Dextrose Agar Medium with Antibiotics, and GP Agar Medium with Antibiotics. For an agar medium that is suffused with fungi, Rose Bengal TS may be added to the agar. Add 5 mL of Rose Bengal TS per liter of agar medium, mix and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. After the agar solidifies, incubate the plates for at least 5 days at between 30°C and 35°C for aerobic bacteria, and between 20°C and 25°C for fungi. If too many colonies are observed, dilute the fluid as described above so that a colony count of not more than 300 per plate may be expected in the case of aerobic bacteria, and not more than 100 per plate in the case of fungi. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

#### 1.3.2. Spread Plate Method

On the solidified and dried surface of the agar medium, pipet 0.05 to 0.2 mL of the test fluid and spread it on the surface with a spreader. The diameter of petri dishes, the kind and volume of the medium to be used, TS to be added, temperature and time of incubation, and the method for calculation of total viable count are the same as described in the Pour Plate Method section.

#### 1.3.3. Serial Dilution Method (Most Probable Number Method)

Prepare tubes each containing 9 to 10 mL of Fluid Casein

Table 5.02-1 Most probable number of microorganisms

Number of tubes in which microbial growth is observed for each quantity of the specimen			Most probable number of microorganisms per g or per mL
0.1 g or 0.1 mL per tube	0.01 g or 0.01 mL per tube	1 mg or 1 $\mu$ L per tube	
3	3	3	> 1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

Soya Bean Digest Medium. To each of the first three tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. If necessary, dilute further. To the last three tubes add 1 mL of the diluent as a control. Incubate the tubes between 30°C and 35°C for not less than 5 days. The control tubes should show no microbial growth. If the reading of the results is difficult or uncertain, transfer about 0.1 mL to a liquid or solid medium and read the results after a further period of incubation between 30°C and 35°C for 24 to 72 hours. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

If, for the first column (0.1 g or 0.1 mL of specimen), the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per mL is likely to be less than 100.

#### 1.3.4. Membrane Filtration Method

This method employs membrane filters of appropriate materials, having a normal pore size not greater than 0.45  $\mu$ m. Filter discs about 50 mm in diameter are recommended, but filters of a different diameter may also be used. Filters, the filtration apparatus, media, etc., should be well sterilized. Usually, take 20 mL of the test fluid (containing 2 g of test specimen), transfer 10 mL of the solution to each of two membrane filters, and filter. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. After the filtration of the test fluid, wash each membrane by filtering through it three or more times with a suitable liquid such as Buffered Sodium Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or the fluid medium to be used. The volume of the washing to be used is approximately 100 mL each time, but if the filter disc is not about 50 mm in diameter, the volume may be adjusted according to the size of the filter. For fatty substances, the

washings may contain a suitable surface-active agent such as Polysorbate 80. Put one of the membrane filters, intended primarily for the enumeration of aerobic bacteria, on the surface of a plate of Casein Soya Bean Digest Agar Medium and the other, intended primarily for the enumeration of fungi, on the surface of a plate of one of Sabouraud-Dextrose Agar Medium with Antibiotics, Potato Dextrose Agar Medium with Antibiotics, and GP Agar Medium with Antibiotics. After incubation of the plates for at least 5 days, at between 30°C and 35°C in the test for the detection of aerobic bacteria and between 20°C and 25°C in the test for fungi, count the number of colonies that are formed. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

#### 1.4. Effectiveness of culture media and confirmation of antimicrobial substances

Use microorganisms of the following strains or their equivalent. Grow them in Casein Soya Bean Digest Agar Medium between 30°C and 35°C for aerobic bacteria and between 20°C and 25°C for *Candida albicans*.

<i>Escherichia coli</i> ,	NBRC 3972, ATCC 8739, NCIMB 8545, etc.
<i>Bacillus subtilis</i> ,	NBRC 3134, ATCC 6633, NCIMB 8054, etc.
<i>Staphylococcus aureus</i> ,	NBRC 13276, ATCC 6538, NCIMB 9518, etc.
<i>Candida albicans</i> ,	NBRC 1393, NBRC 1594, ATCC 2091, ATCC 10231, etc.

Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, pH 7.0, or Phosphate Buffer, pH 7.2 to prepare test suspensions containing 50 to 200 cfu per mL. Growth-promoting qualities are tested by inoculating 1 mL of each microorganism into each medium. The test media are satisfactory if clear evidence of growth appears in all inoculated media after incubation at the indicated temperature for 5 days. When a count of test organisms with a test specimen is less than 1/5 of that without the test specimen, any such effect must be eliminated by dilution, filtration, neutralization or inactivation. To confirm the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total viable count method using sterile Buffered Sodium Chloride-Peptone Solution, pH 7.0 or Phosphate Buffer, pH 7.2 as the control.

#### 2. Test for the detection of specified microorganisms

Enterobacteria and certain other gram-negative bacteria, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*, are included as target strains of the test.

##### 2.1. Sampling and Preparation of the test specimens

Refer to the paragraph on 1.1. Sampling and Preparation of the test specimens.

##### 2.2. Preparation of the test fluid

If necessary, refer to the paragraph on 1.2. Preparation of the test fluid. When test specimens are prepared, use the medium designated in each test, unless otherwise specified. If necessary, eliminate antimicrobial substances so as to permit growth of the inocula, and adjust the quantity of test specimen or increase the volume of medium to suitable values.

#### 2.3. Test Procedure

##### 2.3.1. Enterobacteria and certain other gram-negative bacteria

###### 2.3.1.1. Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid

Lactose Medium to form a suspension or solution. Transfer 10 mL to 90 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium and incubate at between 35°C and 37°C for 18 to 24 hours. Mix by gently shaking the container, take a portion by means of an inoculating loop, and streak it on the surface of Violet Red Bile Glucose Agar. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, the specimen may contain Enterobacteria and certain other gram-negative bacteria.

###### 2.3.1.2. Quantitative evaluation

If Enterobacteria and certain other gram-negative bacteria are found, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Transfer 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Next, transfer 1 mL of the test fluid (containing 0.01 g or 0.01 mL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Furthermore, transfer 1 mL of the test fluid (containing 1 mg or 1 µL of specimen) to a tube containing 9 mL of Fluid Enterobacteria Enrichment Broth-Mossel Medium, and mix. Incubate the tubes at between 35°C and 37°C for 18 to 24 hours, take a portion by means of an inoculating loop, and streak it on the surface of Violet Red Bile Glucose Agar. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, this constitutes a positive result. Note the smallest quantity of the product which gives a positive result and the largest quantity that gives a negative result. Determine from Table 5.02-2 the probable number of microorganisms.

##### 2.3.2. *Escherichia coli*

###### 2.3.2.1. Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to make a suspension or solution. Transfer 1 mL to a fermentation tube containing 9 to 10 mL of Fluid EC Medium and incubate the tube at  $44.5 \pm 0.2^\circ\text{C}$  for  $24 \pm 2$  hours in a water bath. If gas bubbles are not found, the specimen meets the requirements of the test for absence of *Escherichia coli*. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between 30°C and 35°C for 18 to 24 hours. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen and a blue-black appearance under transmitted light, the specimen meets the requirements of the test for absence of *Escherichia coli*. Confirm any suspect colonies on the plate by means of the IMViC test. (Indole production test, Methyl red reaction test, Voges-Proskauer test, and Citrate utilization test); colonies which exhibit the pattern of either [ + + - - ] or [ - + - - ] are judged as *Escherichia coli*. Rapid detection kits for *Escherichia coli* may also be used.

###### 2.3.2.2. Quantitative evaluation

If *Escherichia coli* is found, prepare tubes each containing 9 to 10 mL of Fluid EC Medium. Use three tubes for each dilution. To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium and suspend or dissolve. To each of the first three fermentation tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three fermentation tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three fermentation tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. To the last

three fermentation tubes add 1 mL of the diluent as a control. Incubate the tubes at  $44.5 \pm 0.2^\circ\text{C}$  for  $24 \pm 2$  hours in a water bath. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between  $30^\circ\text{C}$  to  $35^\circ\text{C}$  for 18 to 24 hours. Upon examination, colonies of Gram-negative organisms show both a characteristic metallic sheen and a blue-black appearance under transmitted light. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

**2.3.3. Salmonella**

As in the case of the detection of *Escherichia coli*, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Incubate at between  $30^\circ\text{C}$  to  $35^\circ\text{C}$  for 24 to 72 hours. Examine the medium for growth, and if growth is apparent, mix by gentle shaking, then pipet 1 mL portions, respectively, into 10 mL of Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium, and incubate for 12 to 24 hours. 10 mL of Fluid Selenite-Cystine Medium may be replaced by the same volume of Fluid Rappaport Medium. After the incubation, streak portions from both the fluid media on the surface of at least two of Brilliant Green Agar Medium, XLD Agar Medium, and Bismuth Sulfite Agar Medium, and incubate at between  $30^\circ\text{C}$  and  $35^\circ\text{C}$  for 24 to 48 hours. Upon examina-

tion, if none of the colonies conforms to the description given in Table 5.02-3, the specimen meets the requirements of the test for the absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 5.02-3 are found, transfer suspect colonies individually, by means of an inoculating wire, to a slant of TSI Agar Medium using both surface and deep inoculation. Incubate at between  $35^\circ\text{C}$  and  $37^\circ\text{C}$  for 18 to 24 hours. The presence of genus *Salmonella* is confirmed if, in the deep culture but not in the surface culture, there is a change of color from red to yellow and usually formation of gas with or without production of hydrogen sulfide. Precise identification and typing of genus *Salmonella* may be carried out by using appropriate biochemical and serological tests additionally, including an identification kit.

**2.3.4. Staphylococcus aureus**

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Casein Soya Bean Digest Medium, or another suitable fluid medium without antimicrobial activity, to form a suspension or solution. Incubate the fluid containing the specimen at between  $30^\circ\text{C}$  and  $35^\circ\text{C}$  for 24 to 48 hours, and pipet 1 mL into 9 mL of Fluid Casein Soya Bean Digest Medium with 7.5% sodium chloride. If, upon examination, growth is apparent, use an inoculating loop to streak a portion of the medium on the surface of one of Vogel-Johnson Agar Medium, Baird-Parker Agar Medium, or Mannitol-Salt Agar Medium, and incubate at between  $30^\circ\text{C}$  and  $35^\circ\text{C}$  for 24 to 48 hours. Upon examination, if no colonies of Gram-positive rods having the characteristics listed in Table 5.02-4 are found, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*. Confirm any suspect colonies as *Staphylococcus aureus* by means of the coagulase test. With the aid of an inoculating loop, transfer suspect colonies to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a thermostat at  $37 \pm 1^\circ\text{C}$ . Examine the coagulation after 3 hours and subsequently at suitable intervals up to 24 hours. Test positive and negative controls simultaneously. If no coagulation is observed, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*.

**2.4. Effectiveness of culture media and confirmation of antimicrobial substances**

Grow the test strains listed in Table 5.02-5 in the media indicated at between  $30^\circ\text{C}$  and  $35^\circ\text{C}$  for 18 to 24 hours. Dilute portions of each of the cultures using Buffered Sodium

**Table 5.02-2** Most probable number of microorganisms

Results for each quantity of product			Probable number of microorganisms (CFU per g or per mL)
0.1 g or 0.1 mL	0.01 g or 0.01 mL	1 mg or 1 $\mu\text{L}$	
+	+	+	more than $10^3$
+	+	-	less than $10^3$ and more than $10^2$
+	-	-	less than $10^2$ and more than $10^1$
-	-	-	less than $10^1$

**Table 5.02-3** Morphologic characteristics of *Salmonella* species on selective agar media

Medium	Description of colony
Brilliant Green Agar Medium	Small, transparent and colorless, or opaque, pink or white (often surrounded by a pink to red zone)
XLD Agar Medium	Red, with or without a black center
Bismuth Sulfite Agar Medium	Black or green

**Table 5.02-4** Morphologic characteristics of *Staphylococcus aureus* on selective agar media

Medium	Colonial characteristics
Vogel-Johnson Agar Medium	Black surrounded by a yellow zone
Baird-Parker Agar Medium	Black, shiny, surrounded by a clear zone
Mannitol-Salt Agar Medium	Yellow colonies surrounded by a yellow zone

**Table 5.02-5** Bacteria strains and media used for confirmation of the effectiveness of culture medium and validity of the test for specified microorganisms

Microorganism	Strain number	Media
<i>Escherichia coli</i>	NBRC 3972, ATCC 8739, NCIMB 8545 or equivalent strains	Fluid Lactose Medium
<i>Salmonella</i>	No strain number is recommended*	Fluid Lactose Medium
<i>Staphylococcus aureus</i>	NBRC 13276, ATCC 6538, NCIMB 9518 or equivalent strains	Fluid Casein Soya Bean Digest Medium

\**Salmonella* strains of weak or no pathogenicity may be used. *Salmonella typhi* may not be used.

Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or medium indicated for each bacterial strain to make test suspensions containing about 1000 CFU per mL. As occasion demands, using a mixture of 0.1 mL of each suspension of *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* containing about 1000 CFU, test the validity of the medium and the presence of antimicrobial substances in the presence or absence of the specimen.

### 2.5. Retest

For the purpose of confirming a doubtful result, a retest is conducted using a test specimen 2.5 times the first test specimen. Proceed as directed under Test procedure, but make allowance for the larger specimen size, for example by adjusting the volume of the medium.

### 3. Buffer solution, media and test solution (TS)

Buffer solution, media and TS used for the microbial limit test are described below. Other media may be used if they have similar nutritive ingredients, and selective and growth-promoting properties for the microorganisms to be tested.

#### 3.1. Buffer solution

##### (i) Phosphate Buffer, pH 7.2

For use, dilute the Stock Solution with water in the ratio of 1 to 800, and sterilize at 121°C for 15 to 20 minutes.

Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate in about 500 mL of water. Adjust to pH 7.1 to 7.3 by the addition of 175 mL of sodium hydroxide TS, add water to make 1000 mL, and use this solution as the stock solution. After sterilization by heating in an autoclave, store under refrigeration.

##### (ii) 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 (animal tissue or casein)	1.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.1. Polysorbate 20 or 80 (0.1 to 1.0 w/v%) may be added.

#### 3.2. Media

##### (i) Casein Soya Bean Digest Agar Medium

Pancreatic digest of casein	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

##### (ii) Fluid Casein Soya Bean Digest Medium

Pancreatic digest of casein	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

##### (iii) Sabouraud-Dextrose Agar Medium with Antibiotics

Glucose	40.0 g
Peptone (animal tissue and casein, 1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

##### (iv) Potato Dextrose Agar Medium with Antibiotics

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

##### (v) GP (Glucose-peptone) Agar Medium with Antibiotics

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Peptone	5.0 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.6 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

##### (vi) Fluid Lactose Medium

Meat extract	3.0 g
Gelatin peptone	5.0 g
Lactose monohydrate	5.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. After sterilization, cool immediately.

##### (vii) Fluid EC Medium

Peptone	20.0 g
Lactose monohydrate	5.0 g
Bile salts	1.5 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium chloride	5.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.8 – 7.0. After sterilization cool immediately. If gas remains in durham tube after cool, do not use the tube.

##### (viii) EMB (Eosin-Methylene Blue) Agar Medium

Gelatin peptone	10.0 g
Dipotassium hydrogen phosphate	2.0 g
Lactose monohydrate	10.0 g
Agar	15.0 g

Eosin Y	0.4 g
Methylene blue	65 mg
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.3.

**(ix) Fluid Enterobacteria Enrichment Broth-Mossel Medium**

Gelatin peptone	10.0 g
Glucose monohydrate	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

Mix all the components, boil at 100°C for 30 minutes, and cool immediately. pH after boiling: 7.0 – 7.4.

**(x) Violet Red Bile Glucose Agar**

Yeast extract	3.0 g
Gelatin peptone	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.2 – 7.6. Do not sterilize in an autoclave.

**(xi) Fluid Selenite-Cystine Medium**

Gelatin peptone	5.0 g
Lactose monohydrate	4.0 g
Trisodium phosphate dodecahydrate	10.0 g
Sodium selenite	4.0 g
L-Cystine	10 mg
Water	1000 mL

Mix all the components, and heat to dissolve. Final pH: 6.8 – 7.2. Do not sterilize.

**(xii) Fluid Tetrathionate Medium**

Pancreatic digest of casein	2.5 g
Animal tissue peptone	2.5 g
Sodium deoxycholate	1.0 g
Calcium carbonate	10.0 g
Sodium thiosulfate pentahydrate	30.0 g
Water	1000 mL

Heat the solution of solids to boiling. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 mL of water. Then add 10 mL of a solution of brilliant green (1 in 1000), and mix. Do not heat the medium after adding the brilliant green solution.

**(xiii) Fluid Rappaport Medium**

Soybean peptone	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate	1.6 g
Malachite green oxalate	0.12 g
Magnesium chloride hexahydrate	40.0 g
Water	1000 mL

Dissolve malachite green oxalate and magnesium chloride hexahydrate, and the remaining solids separately in the water, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. For the use, mix the both solutions after sterilization. Final pH: 5.4 – 5.8.

**(xiv) Brilliant Green Agar Medium**

Peptones (animal tissue and casein)	10.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g
Phenol red	80 mg
Brilliant green	12.5 mg
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute. Sterilize just prior to use by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. Cool to about 50°C and pour to petri dishes.

**(xv) XLD (Xylose, Lysine, Deoxycholate) 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
Sodium deoxycholate	2.5 g
Sodium thiosulfate	6.8 g
Ammonium iron (III) citrate	0.8 g
Agar	13.5 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.2 – 7.6. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

**(xvi) Bismuth Sulfite Agar Medium**

Meat extract	5.0 g
Pancreatic digest of casein	5.0 g
Animal tissue peptone	5.0 g
Glucose	5.0 g
Trisodium phosphate dodecahydrate	4.0 g
Iron (II) sulfate heptahydrate	0.3 g
Bismuth sulfite indicator	8.0 g
Brilliant green	25 mg
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.4 – 7.8. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

**(xvii) TSI (Triple Sugar Iron) Agar Medium**

Pancreatic digest of casein	10.0 g
Animal tissue peptone	10.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Ammonium iron (II) sulfate hexahydrate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulfate pentahydrate	0.2 g
Phenol red	25 mg
Agar	13.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. Distribute in small tubes and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5. Use as a slant agar medium. The medium containing 3 g of meat extract or yeast extract additionally, or the medium containing ammonium iron (III) citrate instead of ammo-

nium iron (II) sulfate hexahydrate may be used.

(xviii) Fluid Casein Soya Bean Digest Medium with 7.5% Sodium Chloride

Pancreatic digest of casein	17.0 g
Soybean peptone	3.0 g
Sodium chloride	75.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Add the sodium chloride 70.0 g for (ii) Fluid Casein Soya Bean Digest Medium (containing 5 g of sodium chloride), mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

(xix) Vogel-Johnson Agar Medium

Pancreatic digest of casein	10.0 g
Yeast extract	5.0 g
D-Mannitol	10.0 g
Dipotassium hydrogen phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	25 mg
Agar	16.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute to make solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 – 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xx) Baird-Parker Agar Medium

Pancreatic digest of casein	10.0 g
Meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Agar	20.0 g
Water	950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 – 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxi) Mannitol-Salt Agar Medium

Pancreatic digest of casein	5.0 g
Animal tissue peptone	5.0 g
Bovine meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Phenol red	25 mg
Agar	15.0 g
Water	1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 – 7.6.

### 3.3. Reagents·Test solutions

(i) Amphotericin B powder: Amphotericin B added sodium deoxycholic acid, sterilized by  $\gamma$ -ray.

Amphotericin B TS Dissolve 22.5 mg of amphotericin B

powder in 9 mL of sterile purified water.

(ii) Bile salts: Yellow-brown powder made from dried bile of animal, consist of sodium taurocholic acid and sodium glycocholic acid, and containing not less than 45% of cholic acid. pH of 5% solution: 5.5 – 7.5.

(iii) Rose bengal TS: Dissolve 1 g of rose bengal in water to make 100 mL.

Rose bengal  $C_{20}H_2Cl_4I_4Na_2O_5$  [Special class] Red-brown powder, purple-red solution in water.

(iv) TTC TS: Dissolve 0.8 g of 2,3,5-triphenyl-2H-tetrazolium chlorid in water to make 100 mL, distribute in small tubes, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. Store in light-resistant containers.

### 3.4. Preparation

(i) Preparation of agar medium with TTC: Just prior to use, add 2.5 to 5 mL of TTC TS per liter of sterile agar medium and mix.

(ii) Preparation of agar medium with amphotericin B: Just prior to use, add 2 mL of amphotericin B TS in a liter of agar medium, previously sterilized in an autoclave at 121°C for 15 to 20 minutes, and mix.

(iii) Preparation of agar medium with rose bengal: Add 5 mL of rose bengal TS in a liter of agar medium, and mix. Sterilize in an autoclave at 121°C for 15 to 20 minutes.

## 6. Tests for Preparations

### 6.01 Test for Metal Particles in Ophthalmic Ointments

Test of Metal Particles in Ophthalmic Ointments is a method to test the existence of foreign metal particles in the ophthalmic ointments described in General Rules for Preparations.

#### 1. 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 completely. Allow the samples to cool to room temperature without agitation to solidify the contents. 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.

#### 2. Procedure

Invert each dish on the stage of a suitable microscope previously adjusted to provide not less than 40 times magnifications and equipped with an eyepiece micrometer disk. 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, measuring 50  $\mu$ m or more in any dimension.

Note: Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with the bottom.

#### 3. Evaluation

The preparation complies with the test if the total number

of metal particles of a size equal to or greater than 50  $\mu\text{m}$  found in 10 units tested, is not more than 50, and also the number of dishes containing more than 8 particles is not more than 1. If this requirement is not met, repeat the test with a further 20 units in the same manner, and if the total number of the particles found in the total of 30 units is not more than 150, and also the number of dishes containing more than 8 particles is not more than 3, the preparation complies with the test.

## 6.02 Uniformity of Dosage Units

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ( $\blacklozenge$   $\blacklozenge$ ).

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. The Uniformity of Dosage Units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The uniformity of dosage units can be demonstrated by either of two methods, *Content uniformity* or *Mass variation* (see Table 6.02-1.). The test for *Content Uniformity* of preparations presented in dosage units is based on the assay of the individual contents of drug substance(s) 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:

(i) solutions enclosed in unit-dose containers and into soft capsules  $\blacklozenge$  in which all components are perfectly dissolved $\blacklozenge$ ;

(ii) solids (including powders, granules and sterile solids) that are packaged in single-unit containers and contain no active or inactive added substances;

(iii) solids (including sterile solids) that are packaged in single-unit containers, with or without active or inactive added substances, that have been prepared from true solutions  $\blacklozenge$  in which all components are perfectly dissolved $\blacklozenge$  and freeze-dried in the final containers and are labeled to indicate this method of preparation; and

(iv) 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,  $\blacklozenge$  or in the case of film-coated tablets, the pre-coated tablets, $\blacklozenge$  except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting Content Uniformity requirements.

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 25 mg/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

**Table 6.02-1** Application of Content Uniformity (CU) and Mass Variation (MV) Test for Dosage Forms

Dosage Forms	Type	Sub-type	Dose and ratio of drug substance	
			$\geq 25$ mg & $\geq 25\%$	<25 mg or <25%
Tablets	uncoated		MV	CU
	coated	Film	MV	CU
		others	CU	CU
Capsules	hard		MV	CU
	soft	Sus., eml., gel	CU	CU
		solutions	MV	MV
Solids in single unit containers $\blacklozenge$ (divided forms, lyophilized forms, et al.) $\blacklozenge$	Single component		MV	MV
	Multiple components	Solution freeze-dried in final container	MV	MV
		others	CU	CU
Solutions $\blacklozenge$ (perfectly dissolved) $\blacklozenge$ enclosed in unit-dose containers			MV	MV
Others			CU	CU

Sus.: suspension; eml.: emulsion;

Table 6.02-2

Variable	Definition	Conditions	Value
$\bar{X}$	mean of individual contents ( $x_1, x_2, \dots, x_n$ ) expressed as a percentage of the label claim		
$x_1, x_2, \dots, x_n$	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
$s$	sample standard deviation		$\sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n-1}}$
$RSD$	relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\frac{100s}{\bar{X}}$
$M$ (case 1)  To be applied when $T \leq 101.5$	reference value	If $98.5\% \leq \bar{X} \leq 101.5\%$ , then	$M = \bar{X}$ ( $AV = ks$ )
		If $\bar{X} < 98.5\%$ , then	$M = 98.5\%$ ( $AV = 98.5 - \bar{X} + ks$ )
		If $\bar{X} > 101.5\%$ , then	$M = 101.5\%$ ( $AV = \bar{X} - 101.5 + ks$ )
$M$ (case 2)  To be applied when $T > 101.5$	reference value	If $98.5\% \leq \bar{X} \leq T$ , then	$M = \bar{X}$ ( $AV = ks$ )
		If $\bar{X} < 98.5\%$ , then	$M = 98.5\%$ ( $AV = 98.5 - \bar{X} + ks$ )
		If $\bar{X} > T$ , then	$M = T\%$ ( $AV = \bar{X} - T + ks$ )
Acceptance Value ( $AV$ )			general formula: $ M - \bar{X}  + 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 $0.75M$ while on the high side, no dosage unit result can be greater than $1.25M$ (This is based on an $L2$ value 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 %.		

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 6.02-2.

### 1. 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.

(i) Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).

(ii) Liquid dosage forms: Assay 10 units individually using an appropriate analytical method. 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 6.02-2.).

#### 1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks,$$

in which the terms are as defined in Table 6.02-2.

### 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 substance(s) 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.

(i) 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.

(ii) 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.

(iii) 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.

(iv) 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.

(v) 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.

#### 2.1. Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that ♦the value of  $\bar{X}$  is replaced with *A*, and that ♦the individual contents of the dosage units are replaced with the individual estimated contents defined below.

$x_1, x_2 \dots x_n$  = individual estimated contents of the dosage units tested, where

$$x_i = w_i \times \frac{A}{\bar{W}}$$

$w_1, w_2 \dots w_n$  = individual masses of the dosage units tested,  
*A* = content of drug substance (% of label claim) obtained using an appropriate analytical method.

$\bar{W}$  = mean of individual masses ( $w_1, w_2 \dots, w_n$ ).

### 3. Criteria

Apply the following criteria, unless otherwise specified.

(i) 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 units 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  $(1 + L2 \times 0.01)M$  in *Calculation of Acceptance Value* under *Content Uniformity* or under *Mass Variation*. Unless otherwise specified, *L1* is 15.0 and *L2* is 25.0.

## 6.03 Particle Size Distribution Test for Preparations

Particle Size Distribution Test for Preparations is a method to determine the particle size distribution of preparations described in General Rules for Preparations.

### 1. Procedure

The test is performed employing No. 18 (850  $\mu\text{m}$ ) and No. 30 (500  $\mu\text{m}$ ) sieves with the inside diameter of 75 mm.

Weigh accurately 10.0 g of sample to be tested, 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 remaining on each sieve and in the receiving pan.

## 6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capaci-

ty of a medicine, as a crude material or 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 crude 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).

### 1. Preparation of sample

A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the 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 the sample. Granules in dose-unit 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 the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the 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 the sample. Liquid preparations: shake well, and use as the sample.

### 2. Procedure

Take an amount of the sample so that 'a' in the equation falls between 20 mL and 30 mL, and perform the test.

Accurately weigh the sample of the crude 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  $37 \pm 2^\circ\text{C}$  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  $\langle 2.50 \rangle$  the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination  $\langle 2.54 \rangle$ , end point: pH 3.5). Perform a blank determination.

For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.1 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 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  $37 \pm 2^\circ\text{C}$  for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate  $\langle 2.50 \rangle$  the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination  $\langle 2.54 \rangle$ , end point: pH 3.5). Perform a blank determination.

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

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

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 coefficient of 0.1 mol/L sodium hydroxide VS

t: 1000 mg of crude material or daily dose of preparation (in mg of solid preparation, mL of liquid preparation)  
s: Amount of the sample (in mg of crude material and solid preparation, mL of liquid preparation)

## 6.05 Test for Extractable Volume of Parenteral Preparations

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ( $\blacklozenge$   $\blacklozenge$ ).

$\blacklozenge$ Test for Extractable Volume of Parenteral Preparations 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 declared on the label. The excess volume is determined by the characteristics of the product.  $\blacklozenge$

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 contents. The contents are then cooled to  $20 - 25^\circ\text{C}$  before measuring the volume.

### 1. Single-dose containers

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected 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 standardised dry cylinder (graduated to contain rather than to deliver the designated volumes) 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 2 mL or less 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 10 mL or more 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 2 mL or less, 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 pre-filled syringes

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. 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.

## 6.06 Foreign Insoluble Matter Test for Injections

Foreign Insoluble Matter Test for Injections is a test method to examine foreign insoluble matters in injections.

### 1. Method 1.

This method is applied to either injections in solutions, or vehicles for solid injections to be dissolved before use.

Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 lx under an incandescent lamp: Injections or vehicles 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 lx, with an incandescent lamp at appropriate distances above and below the container.

### 2. Method 2.

This method is applied to solid injections to be dissolved before use.

Clean the exterior of containers, and dissolve the contents with vehicles 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 is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 lx, right under an incandescent lamp.

## 6.07 Insoluble Particulate Matter Test for Injections

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Insoluble particulate matters in injections and parenteral

infusions consist of extraneous, mobile undissolved particles, other than gas bubbles, that are unintentionally present 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. When examining injections and parenteral infusions for sub-visible particles, Method 1 is preferably applied. However, it may be necessary to test some preparations by Method 1 followed by Method 2 to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for sub-visible particles 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, the test should be carried out according to Method 2. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. 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 obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterise the level of particulate contamination in a large group of units.

### 1. Method 1. Light Obscuration Particle Count Test

#### 1.1. 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. ♦

#### ♦1.1.1. 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  $\mu\text{m}$  in diameter (PSL particles) in mono-dispersed suspension. The PSL particles should have either a domestic or international traceability in terms of length, with a level of uncertainty at not greater than 3%. The particles to be used for calibration should be dispersed in *particle-free water*.

##### 1.1.1.1. Manual method

The particle size response of the system to be applied should be 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  $\pm 20\%$  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  $\mu\text{m}$  of the apparatus is obtained.

##### 1.1.1.2. Electronic method

In the use of multichannel peak height analyzer, the parti-

cle 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  $\mu\text{m}$  of the apparatus is obtained. In this case, the instrument manufacturer or the user should validate the obtainability of the same result as that of the manual method.

#### 1.1.1.3. 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 manufacturer or the user should validate the obtainability of the same result as that of the manual method.

#### 1.1.2. Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case the decrease of test solution is measured by the mass method after measuring the test solution of 10 mL.

#### 1.1.3. Sample flow rate

The flow rate of the sample indicated into the sensor should be calculated from the observed sample volume and time, and should be conformed within the range of the manufacturer's specification for sensor used.

#### 1.1.4. Sensor

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 threshold accuracy also needs to be confirmed. Testing should accordingly be performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PSL spheres having mean diameter of approximately 10  $\mu\text{m}$ , of a concentration at 1000 particles/mL  $\pm 10\%$ , not more than 5% of CV value).

During measurement, stirring should be made for assuring the uniformity in sample concentration.

##### 1.1.4.1. Sensor resolution (Particle size resolution of apparatus)

Measurement should be made by either one of the following methods. The difference between the threshold of particle size counting 16% and 84% of the total counts and the test-particle size should be within 10%, whereas, electronic method and automated method should be both validated for obtaining the same result as that of the manual method.

(i) Manual method to obtain the spread of histogram prepared from the counting value of the apparatus.

(ii) Electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer.

(iii) 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.

##### 1.1.4.2. Particle counting accuracy

Data obtained by counting particles of 5  $\mu\text{m}$  and greater should be 763 to 1155 particles per 1 mL.

##### 1.1.4.3. Threshold accuracy

Particle size calculated from a threshold corresponding to 50% counts for particles of 5  $\mu\text{m}$  and greater should fall within  $\pm 5\%$  of the mean diameter of the test particles. ♦

#### 1.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash 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 5 mL, according to the method described below. If the number of particles of 10  $\mu\text{m}$  or greater size exceeds 25 for the combined 25 mL, 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.

#### 1.3. Method

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

Powders for parenteral use 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 large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

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

#### 1.4. Evaluation

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

*Test 1.A—Solutions for injection supplied in containers with a nominal content of ♦equal to or ♦, more than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per milliliter equal to or greater than 10  $\mu\text{m}$  and does not exceed 3 per milliliter equal to or greater than 25  $\mu\text{m}$ .

*Test 1.B—Solutions for injection supplied in containers with a nominal content of less than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than  $10\ \mu\text{m}$  and does not exceed 600 per container equal to or greater than  $25\ \mu\text{m}$ .

## 2. Method 2. Microscopic Particle Count Test

### 2.1. 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, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to  $100 \pm 10$  magnifications. The ocular micrometer is a circular diameter graticule (see Fig. 6.07-1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles  $10\ \mu\text{m}$  and  $25\ \mu\text{m}$  in diameter at 100 magnifications, and a linear scale graduated in  $10\ \mu\text{m}$  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  $\pm 2$  per cent is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focussable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of  $10^\circ$  to  $20^\circ$ .

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  $1.0\ \mu\text{m}$  or finer in nominal pore size.

### 2.2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to re-

move 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 50 mL volume of *particle-free water* according to the method described below. If more than 20 particles  $10\ \mu\text{m}$  or larger in size or if more than 5 particles  $25\ \mu\text{m}$  or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

### 2.3. Method

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 large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units is 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 25 mL with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted 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 large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliter of *particle-free water*. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If needed add stepwise a portion of the solution until the entire volume is filtered. After the last addition of solution, 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 equal to or greater than  $10\ \mu\text{m}$  and the number of particles that are equal to or greater than  $25\ \mu\text{m}$ . 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  $10\ \mu\text{m}$  and  $25\ \mu\text{m}$  graticule reference circles. Thereby the

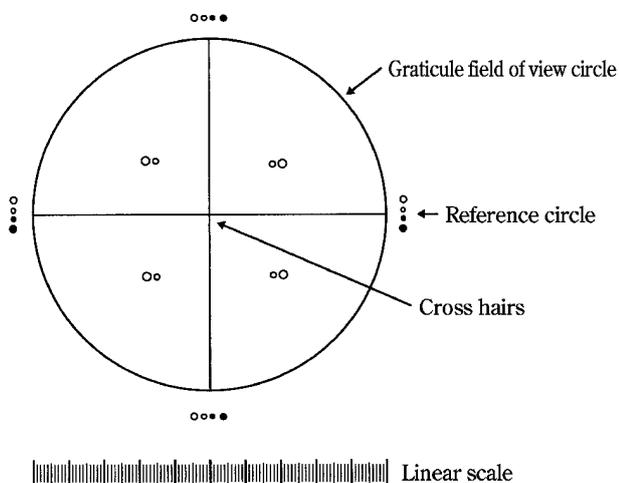


Fig. 6.07-1 Circular diameter graticule

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.

#### 2.4. Evaluation

*Test 2.A—Solutions for injection supplied in containers with a nominal content of ♦equal to or ♦ more than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per milliliter equal to or greater than 10  $\mu\text{m}$  and does not exceed 2 per milliliter equal to or greater than 25  $\mu\text{m}$ .

*Test 2.B—Solutions for injection supplied in containers with a nominal content of less than 100 mL*

The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than 10  $\mu\text{m}$  and does not exceed 300 per container equal to or greater than 25  $\mu\text{m}$ .

#### ♦3. Reagents

*Particle-free water:* The filtered water through a membrane filter with a pore size not exceeding 0.45  $\mu\text{m}$ , containing not more than 5 particles of 10  $\mu\text{m}$  or greater size, and not more than 2 particles of 25  $\mu\text{m}$  or greater size in 10 mL of the insoluble particle number measured by the light obscuration particle counter. ♦

## 6.08 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.

### 1. Apparatus

Use 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 incapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.

(iii) Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10  $\mu\text{m}$  in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble par-

ticulate matter equal to or greater than 25  $\mu\text{m}$  in size should not be found on the filter. When necessary, wash the filter with water for particulate matter test.

### 2. Reagents

(i) Water for particulate matter test: Water prepared before use by filtering through a membrane filter with a pore size not exceeding 0.45  $\mu\text{m}$ . It contains not more than 10 particles of 10  $\mu\text{m}$  or greater size in 100 mL.

### 3. Procedure

#### 3.1. Aqueous ophthalmic solutions

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with water for particulate matter test, and filter under reduced pressure with 200 mL of water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a downlight from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150  $\mu\text{m}$  within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of water for particulate matter test. Clean the outer surface of the container, and mix the sample solution gently by inverting the container several times. Remove the cap, clean the outer surface of the nozzle, and pour the sample solution into a measuring cylinder which has been rinsed well with water for particulate matter test. Repeat the process to prepare 25 mL of the test solution. Pour the test solution into the filter holder along the inner wall of the holder. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solution, dilute suitably with water for particulate matter test or suitable diluent and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of water for particulate matter test or suitable diluent in such manner as to wash the inner wall of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or larger than 300  $\mu\text{m}$  within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

#### 3.2. Ophthalmic solutions which are dissolved before use

Proceed as directed in Aqueous Ophthalmic Solutions after dissolving the sample with the constituted solution.

#### 3.3. Suspension type ophthalmic solutions

Proceed as directed in Aqueous Ophthalmic Solutions.

Take 25 mL of the sample in a vessel, which has been rinsed well with water for particulate matter test, add a suitable amount of a suspension-solubilizing solvent or an adequate solvent, shake to dissolve the suspending particles, and use this solution as the sample solution. Use a membrane filter which is not affected by the solvent to be used.

### 3.4. Ophthalmic solutions contained in a single-dose container

Proceed as directed in Aqueous Ophthalmic Solutions, using 10 samples for the test. A 13-mm diameter membrane filter and a 4-mm diameter filter holder for retaining insoluble particulate matter are used.

### 4. Evaluation

The preparation complies with the test if the calculated number per mL of insoluble particles of a size equal to or greater than  $300\ \mu\text{m}$  is not more than 1.

## 6.09 Disintegration Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols ( $\blacklozenge$   $\blacklozenge$ ).

Disintegration Test is provided to determine whether tablets, capsules,  $\blacklozenge$ granules, dry syrups or pills,  $\blacklozenge$  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 constituent.

### 1. 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  $35^\circ$  and  $39^\circ$ , 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 the time required for the downward stroke, and 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.

(i) **Basket-rack assembly:** The basket-rack assembly consists of six open-ended transparent tubes, each  $77.5 \pm 2.5$  mm long and having an inside diameter of 20.7 to 23 mm and a wall 1.0 to 2.8 mm thick; the tubes are held in a vertical position by two 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 aper-

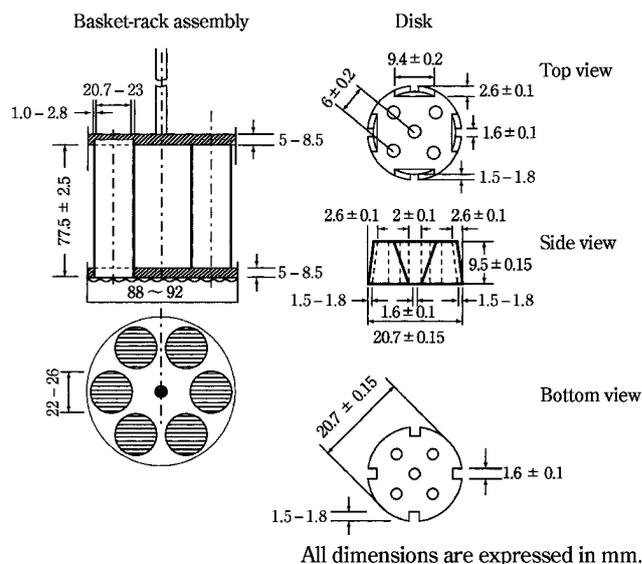
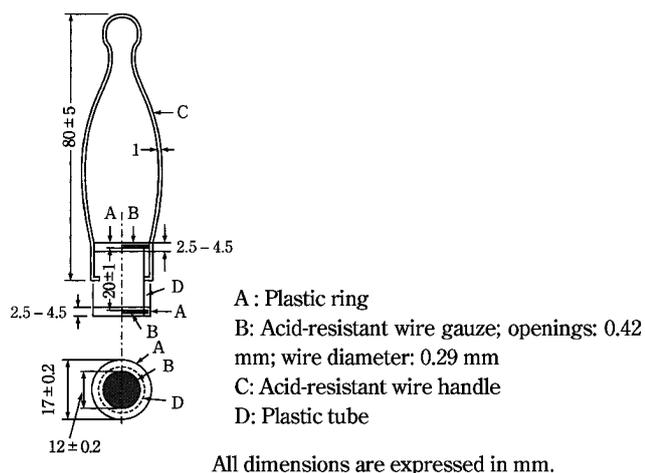


Fig. 6.09-1 Disintegration apparatus

tures 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. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis. The basket-rack assembly conforms to the dimensions found in Fig. 6.09-1. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained:  $\blacklozenge$  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.  $\blacklozenge$

(ii) **Disks:** The use of disks is permitted only where specified or allowed. Each tube is provided with a cylindrical disk  $9.5 \pm 0.15$  mm thick and  $20.7 \pm 0.15$  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  $2 \pm 0.1$  mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered  $6 \pm 0.2$  mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes 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  $1.6 \pm 0.1$  mm, and its bottom edges lie at a depth of 1.5 - 1.8 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of  $9.4 \pm 0.2$  mm, and its center lies at a depth of  $2.6 \pm 0.1$  mm from the cylinder's 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 Proce-



◆Fig. 6.09-2 Auxiliary tube.◆

ture. The disks conform to dimensions found in Fig. 6.09-1. 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.

◆(iii) Auxiliary tube: The auxiliary tube, as illustrated in Fig. 6.09-2, consists of a plastic tube D,  $12 \pm 0.2$  mm in inside diameter,  $17 \pm 0.2$  mm in outside diameter,  $20 \pm 1$  mm in length, having both outside ends screw-cut, and two plastic rings A, each  $12 \pm 0.2$  mm in inside diameter,  $17 \pm 0.2$  mm in outside diameter,  $2.5 - 4.5$  mm in length, having one inside end screw-cut. Acid-resistant 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  $20 \pm 1$  mm. A handle of an acid-resistant wire, 1 mm in diameter and  $80 \pm 5$  mm in length, is attached to the mid portion of the plastic tube. The auxiliary tube is used for the test of granules and capsules containing enteric coated granules.◆

## 2. Procedure

### 2.1. Immediate-release preparations

In case of tablets, capsules ◆and pills (except for pills containing crude 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 as the immersion fluid,◆ maintained at  $37 \pm 2^\circ\text{C}$  as the immersion fluid. ◆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. ◆Complete disintegration is defined as that state in which any residue of the unit, except fragments of 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 crude drugs, carry out the test for 60

minutes in the same manner, using 1st fluid for disintegration test as the immersion fluid. When any residue of the unit is observed, carry out the test successively for 60 minutes, using 2nd fluid for disintegration test.◆

◆In case of granules and dry syrups, shake preparations on a No. 30 ( $500 \mu\text{m}$ ) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, 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 as the immersion fluid, maintained at  $37 \pm 2^\circ\text{C}$  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. Complete disintegration is 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.◆

### ◆2.2. Enteric coated preparations

Unless otherwise specified, perform the following two tests, (a) the test with 1st fluid for disintegration test and (b) the test with the 2nd fluid for disintegration test, separately.

#### 2.2.1. Enteric coated tablets and capsules

(i) The test with 1st fluid for disintegration test: Carry out the test for 120 minutes, using 1st fluid for disintegration test 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 six 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.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new dosage units for 60 minutes, using 2nd fluid for disintegration test and determine if the test is met or not.

#### 2.2.2. Enteric coated granules and capsules containing the enteric coated granules

Shake granules or contents taken out from capsules on a No. 30 ( $500 \mu\text{m}$ ) sieve as directed in Particle Size Distribution Test for Preparations <6.03>, 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 1st and 2nd fluids for disintegration test.

(i) The test with 1st fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test for 60 minutes, using 1st fluid for disintegration test. The test is met if particles fallen from the openings of the wire gauze number not more than 15.

(ii) The test with 2nd fluid for disintegration test: According to the procedure described in immediate-release preparations, carry out the test with new samples for 30 minutes, using 2nd fluid for disintegration test and determine if test is met or not.◆

## 6.10 Dissolution Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Dissolution Test is provided to determine compliance with the dissolution requirements for dosage forms administered orally. ♦ This test also aims at preventing significant bioequivalence. ♦ In this test, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified equivalent to minimum dose.

### 1. Apparatus

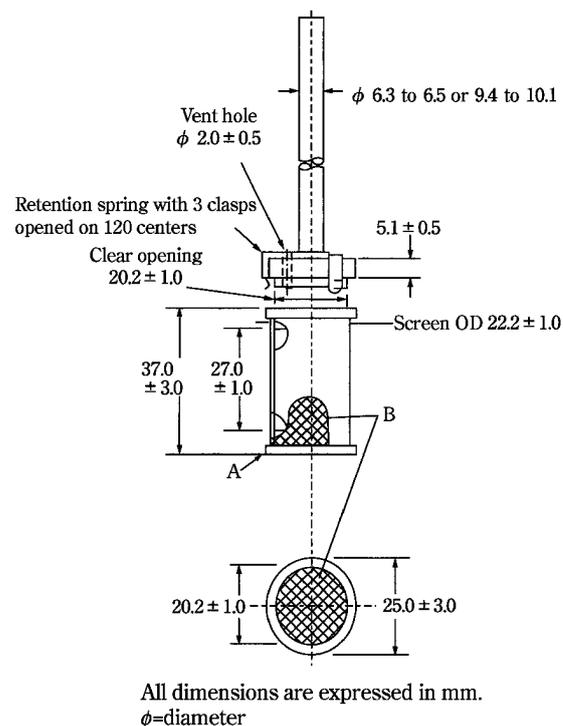
#### 1.1. Apparatus for Basket Method (Apparatus 1)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material\*<sup>1</sup>; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at  $37 \pm 0.5^\circ\text{C}$  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.\*<sup>2</sup> The shaft is positioned so that its axis 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  $\pm 4\%$ .

Shaft and basket components of the stirring element shown in Fig. 6.10-1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about 0.0001 inch ( $2.5\ \mu\text{m}$ ) 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  $25 \pm 2$  mm during the test.

#### 1.2. Apparatus for 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 paddle conforms to the specifications shown in Fig. 6.10-2. The distance of  $25 \pm 2$  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 provided 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



- A: Note—Maximum allowable runout at “A” is  $\pm 1.0$  mm when the part is rotated on center line axis with basket mounted.
- B: Screen with welded seam, 0.25–0.31 mm wire diameter with wire openings of 0.36–0.44 mm [Note—After welding, the screen may be slightly altered.]

Fig. 6.10-1 Apparatus 1, Basket stirring element

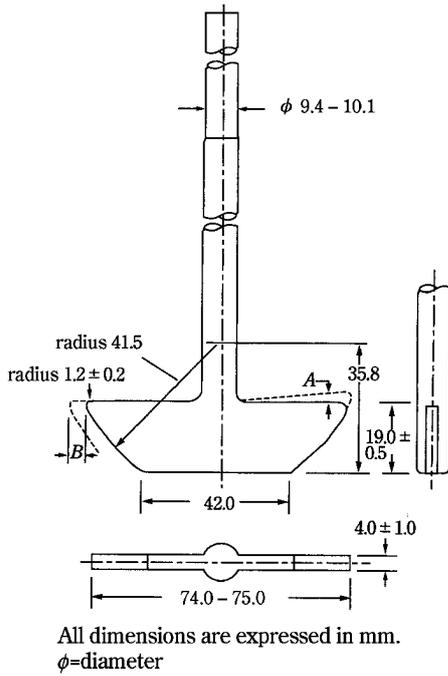
♦ usually ♦ allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material, such as not more than a few turns of wire helix or such one shown in Fig. 6.10-2a, may be attached to the dosage unit that would otherwise float. Other validated sinker devices may also be used. ♦ If the use of sinker is specified, unless otherwise specified, use the sinker device shown in Fig. 6.10-2a. ♦

#### 1.3. Apparatus for 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  $37 \pm 0.5^\circ\text{C}$ . 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 16 mL per minute, with standard flow rates of 4, 8, and 16 mL per minute. It must deliver a constant flow ( $\pm 5$  per cent of the nominal flow rate); the flow profile should be sinusoidal with a pulsation of  $120 \pm 10$  pulses per minute. A pump without the pulsation may also be used. Dissolution test procedure using the flow-through cell must be characterized with respect to rate and any pulsation.

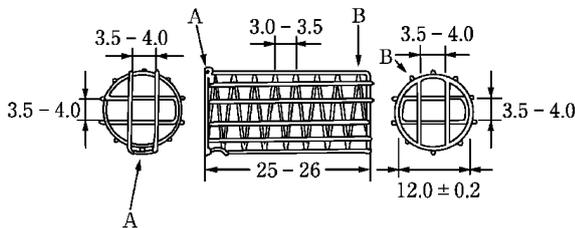
The flow-through cell (see Figures 6.10-3 and 6.10-4), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph) that prevents escape of undissolved particles from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom



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  $\pm 1.0$  mm unless otherwise stated.

Fig. 6.10-2 Apparatus 2, Paddle stirring element



- A: Acid-resistant wire clasp
- B: Acid-resistant wire support

Fig. 6.10-2a Alternative sinker

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 6.10-3 and 6.10-4) is available for positioning of special dosage forms. The cell is immersed in a water bath, and the temperature is maintained at  $37 \pm 0.5^\circ\text{C}$ .

The apparatus uses a clamp mechanism of two O-rings 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.

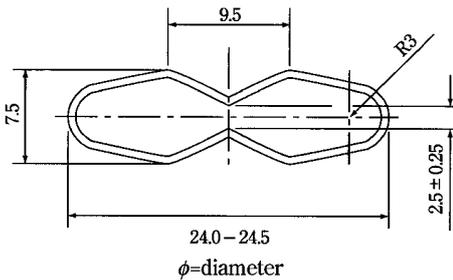
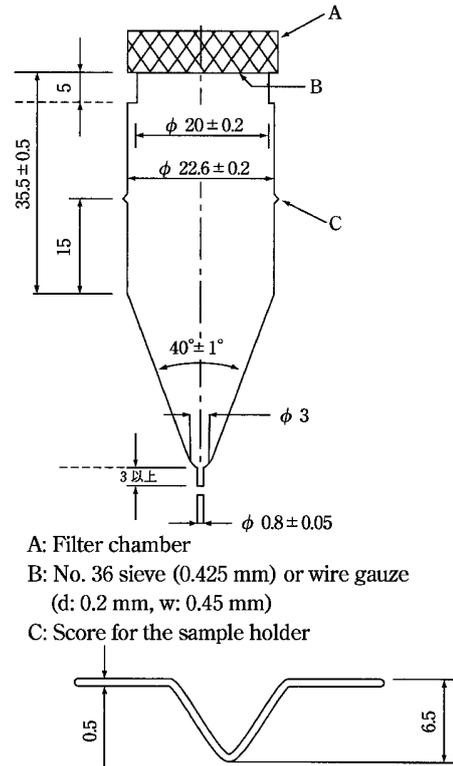


Fig. 6.10-3 Apparatus 3

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

2. 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.

3. Procedure

3.1. Basket Method or Paddle Method

3.1.1. Immediate-release Dosage Forms

(i) Procedure: Place the stated volume of the dissolution medium ( $\pm 1\%$ ) in the vessel of the specified apparatus, assemble the apparatus, equilibrate the dissolution medium to

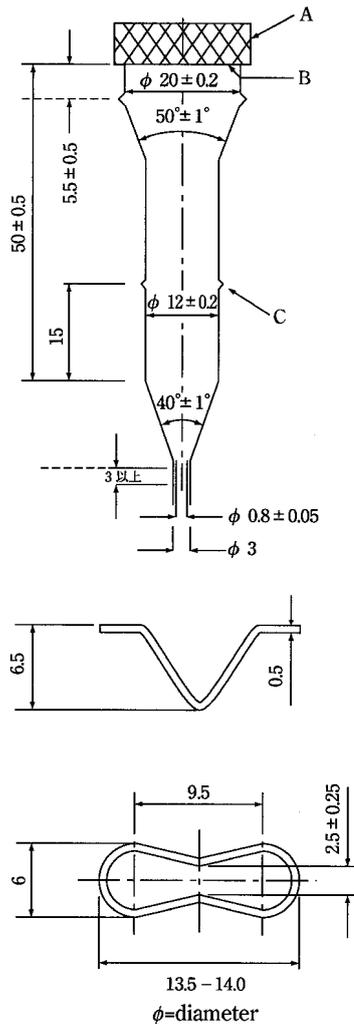


Fig. 6.10-4 Apparatus 3

Small cell for tablets and capsules (top); tablet holder for the small cell (bottom)

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

$37 \pm 0.5^\circ\text{C}$ , 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 specified 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 withdrawn for analysis with equal volumes of fresh Dissolution Medium at  $37^\circ\text{C}$  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.\*<sup>3</sup> 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 nec-

essary.

(ii) **Dissolution Medium:** An appropriate dissolution medium is used. The volume specified refers to measurements made between  $20^\circ\text{C}$  and  $25^\circ\text{C}$ . If the dissolution medium is a 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 results of the test. If dissolved gases influence the dissolution results, remove dissolved gases prior testing.\*<sup>4</sup>]

(iii) **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  $\pm 2\%$ .

### 3.1.2. Extended-release Dosage Forms

(i) **Procedure:** Proceed as described for Immediate-Release Dosage Forms.

(ii) **Dissolution Medium:** Proceed as directed under Immediate-Release Dosage Forms.

(iii) **Time:** The test-time points, generally three, are expressed in hours.

### 3.1.3. Delayed-release Dosage Forms

(i) **Procedure:** Unless otherwise specified, proceed the acid stage test and buffer stage test separately as described for Immediate-Release Dosage Forms.♦

(ii) **Dissolution Medium:** Acid stage: Unless 1st fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms. Buffer stage: Unless 2nd fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms.♦

(iii) **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  $\pm 2\%$ , unless otherwise specified.

## 3.2. Flow-Through Cell Method

### 3.2.1. Immediate-release Dosage Forms

(i) **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  $37 \pm 0.5^\circ\text{C}$  through the bottom of the cell to obtain the flow rate specified and measured with an accuracy of 5%. Collect the eluate by fractions at each of the times stated. Perform the analysis as directed. Repeat the test with additional dosage units.

(ii) **Dissolution Medium:** Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

(iii) **Time:** Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

### 3.2.2. Extended-release Dosage Forms

(i) **Procedure:** Proceed as described for Immediate-Release Dosage Forms under Flow-Through Cell Method.

(ii) **Dissolution Medium:** Proceed as described for Immediate-Release under Flow-Through Cell Method.

(iii) **Time:** The test-time points, generally three, are expressed in hours.

## 4. Interpretation

### 4.1. Immediate-release Dosage Forms

♦Follow Interpretation 1 when the value  $Q$  is specified in

Acceptance Table 6.10-1

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 $Q - 25\%$ .

Acceptance Table 6.10-2

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.

the individual monograph, otherwise follow Interpretation 2.♦

#### 4.1.1. 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 6.10-1. 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.

#### 4.1.2. ♦ 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 another 6 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 6.10-3

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.

Acceptance Table 6.10-4

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 $Q - 25\%$ .

## 4.2. Extended-release Dosage Forms

### 4.2.1. ♦ 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 6.10-2. Continue testing through the three levels unless the results conform at either L1 or L2. Limits on the amounts of active ingredient dissolved are expressed in terms of the percentage of 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.

### 4.2.2. ♦ 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 another 6 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.♦

## 4.3. Delayed-release Dosage Forms

♦ Follow Interpretation 1 when the value  $Q$  is specified in the test using 2nd fluid for dissolution test in the individual monograph, otherwise follow Interpretation 2.

### 4.3.1. Interpretation 1

(i) Test using 1st fluid 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 6.10-3. Continue testing through the three levels unless the result conforms at A2.♦

(ii) ♦ Test using 2nd fluid for dissolution test♦: Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the units tested con-

form to Acceptance Table 6.10-4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of  $Q$  in Acceptance Table 6.10-4 is the amount ♦specified in monograph♦ of active ingredient dissolved, expressed as a percentage of the labeled content. The 5% and 15% and 25% values in Acceptance Table 6.10-4 are percentages of the labeled content so that these values and  $Q$  are in the same terms.

#### 4.3.2. ♦Interpretation 2

Unless otherwise specified, both the tests using 1st fluid for dissolution test and 2nd fluid 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 another 6 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. ♦

\*<sup>1</sup>The materials should not sorb, react, or interfere with the specimen being tested.

\*<sup>2</sup>If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

\*<sup>3</sup>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.

\*<sup>4</sup>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.

## 6.11 Foreign Insoluble Matter Test for Ophthalmic Solutions

Foreign Insoluble Matter Test for Ophthalmic Solutions is a test method to examine foreign insoluble matters in ophthalmic solutions.

When inspect with the unaided eyes at a position of luminous intensity of 3000 – 5000 lx under an incandescent lamp after cleaning the exterior of containers, Ophthalmic Solutions must be clear and free from readily detectable foreign insoluble matters.

## 7. Tests for Containers and Packing Materials

### 7.01 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.

(1) The containers are colorless or light brown and transparent, and have no bubbles which interfere the test of the Foreign Insoluble Matter Test for Injections <6.06>.

(2) 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 of the test for Rubber Closure for Aqueous Infusions <7.03>.

(3) 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.

(i) 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 100 mL.

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 μm) sieve. Combine all portions of the glass powder passed through a No. 12 (1400 μm) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850 μm) and No. 50 (300 μm) sieves. Transfer 7 g of the powder, which has passed through a No. 18 (850 μm) sieve but not through a No. 50 (300 μm) sieve to a No. 50 (300 μm) 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 to cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder thus prepared to a 200-mL conical 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 conical flask of hard glass, wash well the residual powdered glass with three 20-mL portions of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate <2.50> 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	

syringes used as containers) 2.00 mL

(ii) Method 2: This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100 mL.

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 falls to room temperature, measure exactly 100 mL of the this solution, and transfer to a 250-mL conical flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate <2.50> 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 conical 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.

(4) 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 40 mL of this solution according to Method 1 of the Iron Limit Test <1.10>, and perform the test according to Method B. Prepare the control solution with 2.0 mL of the Standard Iron Solution.

(5) 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 not less than 1.0 mm, the percent transmissions between 590 nm and 610 nm are not less than 45%.

## 7.02 Test Methods for Plastic Containers

Test methods for plastic containers may be used for designing and assuring quality 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 numbers and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered if necessary.

It is not allowable for plastic containers for the aqueous injections to interact with the pharmaceutical contained therein resulting in the deterioration of its efficacy, safety or

stability, and to contaminate with microorganisms. They should meet the requirements prescribed in 2. Requirements for Plastic Containers for Aqueous Injections.

### 1. Test methods

#### 1.1. Combustion tests

##### 1.1.1. Residue on ignition

Weigh accurately about 5 g of cut pieces of the container and perform the test according to Residue on Ignition <2.44>.

##### 1.1.2. 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 Heavy Metals Limit Test <1.07>. Prepare the control solution with 2.0 mL of Standard Lead Solution.

##### 1.1.3. Lead

###### 1.1.3.1. 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 water bath, 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 monohydrate (1 in 2) and hydrochloric acid (1:1), and add 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the obtained filtrate add 10 mL of a solution of diammonium hydrogen citrate (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 *N,N*-diethyldithiocarbamate trihydrate (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 if necessary, and use the layer as the sample solution. Separately, to 2.0 mL of Standard Lead Solution add water to make exactly 10 mL. To 1.0 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 sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of lead in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Lead hollow-cathode lamp.

Wavelength: 283.3 nm.

###### 1.1.3.2. 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 0.1 mL 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 5 mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of 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 10 mL 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 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 *N,N*-diethyldithiocarbamate trihydrate (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 sample solution. Separately, pipet 5 mL of Standard Lead Solution, add water to make exactly 50 mL, 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 sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in Method 1, and determine the concentration of lead in the sample solution.

#### 1.1.4. Cadmium

##### 1.1.4.1. Method 1

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in "1.1.3.1. Method 1", and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in "1.1.3.1. Method 1" and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of cadmium in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

##### 1.1.4.2. Method 2

To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in "1.1.3.2. Method 2", and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in "1.1.3.2. Method 2" and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in "1.1.4.1. Method 1", and determine the concentration of cadmium in the sample solution.

#### 1.1.5. 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 dropwise 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 5 mL of this solution into a 25-mL volumetric flask (A). Transfer the remaining solution to a 25-mL beaker (B) by washing out with 10 mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia solution (28) (1 in 2), and measure the volume consumed for neutralization as *a* mL. To the volumetric flask, A, add potassium permanganate TS dropwise 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 monohydrate (1 in 10), *a* mL of diluted ammonia solution (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 sample solution. Separately, pipet 1.0 mL of Standard Tin Solution, add 5 mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and the standard solution according to Ultraviolet-visible Spectrophotometry <2.24> at 510 nm, using water as the blank.

#### 1.2. 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 cm<sup>2</sup> when the thickness is 0.5 mm or less, or about 600 cm<sup>2</sup> 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:

(i) Foaming test: Place 5 mL 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.

(ii) pH <2.54>: 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.

(iii) Potassium permanganate-reducing substances: Place

20.0 mL of the test solution in a glass-stoppered, conical flask, add 20.0 mL of 0.002 mol/L potassium permanganate VS and 1 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate <2.50> 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 0.002 mol/L potassium permanganate VS between these solutions.

(iv) UV spectrum: Read 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 Ultraviolet-visible Spectrophotometry <2.24>.

(v) Residue on evaporation: Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying at 105°C for 1 hour.

### 1.3. Test for fine particles

#### 1.3.1. Test procedure

Rinse thoroughly the inside and outside of containers to be tested with water for particulate matter test, fill the container with the labeled volume of water for particulate matter test or 0.9 w/v% sodium chloride solution, adjust the amount of air in the container to about 50 mL per 500 mL of the labeled volume, put tight stopper to the container, and heat it at 121°C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container from the autoclave, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121°C, employ a suitable temperature-time combination as directed under 1.2. Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle of filterless infusion set into the container through the rubber closure of the container, take the effluent from the container while mixing gently in a clean container for measurement, and use it as the test solution.

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  $\mu\text{m}$  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 particulate matter test or 0.9 w/v% sodium chloride solution.

Count five times the numbers of particles with diameters of 5 – 10  $\mu\text{m}$ , 10 – 25  $\mu\text{m}$  and more than 25  $\mu\text{m}$  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.

#### 1.3.2. Reagent

Water for particulate 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 – 10  $\mu\text{m}$  in size per 1.0 mL.

### 1.4. Transparency test

#### 1.4.1. Method 1

This method can only be applied to containers which have a smooth and not embossed surface and rather low curvature. Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces of about 0.9 × 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 as directed under Ultraviolet-visible Spectrophotometry <2.24> using a cell filled with water as a blank.

#### 1.4.2. Method 2

Sensory test—This method 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.

##### 1.4.2.1. Test solutions

(i) Formadin standard suspension: To 15 mL of the formadin stock suspension add water to make 1000 mL. Use within 24 hours of preparation. Shake thoroughly before use.

(ii) Reference suspension: To 50 mL of Formadin standard suspension add water to make 100 mL.

##### 1.4.2.2. Test procedures

(i) Method 2A (with control): Take two of containers to be tested, and fill one of them with the labeled volume of the reference suspension and the other with the same volume of water. Show these two containers to five subjects, separately, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) Method 2B (without control): Take six of containers to be tested, put number to each of them, and fill three of them with the labeled volume of the reference suspension and the others with the same volume of water. Show each one of these containers at random order to five subjects, separately, ask if it is turbid or not, and calculate the percentage of the answer judged as “turbid” (100 X/15, X: number of containers judged as “turbid”) for reference suspension-filled containers group and water-filled containers group, respectively.

### 1.5. Water vapor permeability test

#### 1.5.1. 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.

#### 1.5.2. 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.

##### 1.5.2.1. 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.

##### 1.5.2.2. 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 Table 7.02-1. Add desiccant to 10 of the containers, designated test containers, 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

**Table 7.02-1** Torque Applicable to Screw-Type Container

Closure Diameter (mm)	Torque (N·cm)
8	59
10	60
13	88
15	59 – 98
18	78 – 118
20	88 – 137
22	98 – 157
24	118 – 206
28	137 – 235
30	147 – 265
33	167 – 284
38	196 – 294
43	196 – 304
48	216 – 343
53	235 – 402
58	265 – 451
63	284 – 490
66	294 – 510
70	314 – 569
83	363 – 735
86	451 – 735
89	451 – 794
100	510 – 794
110	510 – 794
120	618 – 1069
132	677 – 1069

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 1 mg if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at  $75 \pm 3\%$  relative humidity and a temperature of  $20 \pm 2^\circ\text{C}$ . 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, in mL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

$$(1000/14 V) [(T_f - T_i) - (C_f - C_i)]$$

$V$ : 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)

#### 1.6. 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<sup>2</sup> at

20°C for 10 minutes. Judge the leakiness by observing the color of the paper.

#### 1.7. 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.

##### 1.7.1. Cell lines

The recommended cell lines are L929 (American Type Culture Collection-ATCC CCL1) and V79 (Health Science Research Resources Bank-JCRB0603). In addition, 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 and V79 cells.

##### 1.7.2. Culture medium

Eagle's minimum essential medium prepared as follows shall be used. Dissolve the chemicals listed below in 1000 mL of water. Sterilize the solution by autoclaving at 121°C for 20 minutes. Cool the solution to room temperature, and add 22 mL of sterilized sodium hydrogen carbonate TS and 10 mL of sterilized glutamine TS. To the resultant solution add fetal calf serum (FCS) to make 10 vol% FCS in the medium.

sodium chloride	6.80 g
potassium chloride	400 mg
sodium dihydrogen phosphate (anhydrous)	115 mg
magnesium sulfate (anhydrous)	93.5 mg
calcium chloride (anhydrous)	200 mg
glucose	1.00 g
L-arginine hydrochloride	126 mg
L-cysteine hydrochloride monohydrate	31.4 mg
L-tyrosine	36.0 mg
L-histidine hydrochloride monohydrate	42.0 mg
L-isoleucine	52.0 mg
L-leucine	52.0 mg
L-lysine hydrochloride	73.0 mg
L-methionine	15.0 mg
L-phenylalanine	32.0 mg
L-threonine	48.0 mg
L-tryptophan	10.0 mg
L-valine	46.0 mg
succinic acid	75.0 mg
succinic acid hexahydrate	100 mg
choline bitartrate	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
biotin	0.02 mg
phenol red	6.0 mg

##### 1.7.3. Reagents

(i) Sodium hydrogen carbonate TS: Dissolve 10 g of sodium hydrogen carbonate in water to make 100 mL. 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.

(ii) Glutamine TS: Dissolve 2.92 g of L-glutamine in water to make 100 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than  $0.22\ \mu\text{m}$ .

(iii) Phosphate buffer solution (PBS): Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogenphosphate, 8.00 g of sodium chloride, and 1.15 g of disodium hydrogenphosphate (anhydrous) in water to make 1000 mL. Sterilize the solution by autoclaving at  $121^\circ\text{C}$  for 20 minutes.

(iv) Trypsin TS: Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution to make 1000 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than  $0.22\ \mu\text{m}$ .

(v) Formaldehyde solution: Dilute formaldehyde solution with water by a factor of ten.

(vi) Giemsa's stain solution: Dilute a commercially available Giemsa's test solution with the diluent by a factor of fifty. Prepare before use.

(vii) Diluent: Dissolve 4.54 g of potassium dihydrogenphosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL.

#### 1.7.4. Devices and instruments

(i) Pipets: Pasteur pipet, pipet for partial delivery, measuring pipet for partial delivery, and dispenser with microtip.

(ii) Screw-capped glass bottles: 50 – 1000 mL volume.

(iii) Sterile disposable centrifuge tubes: 15 and 50 mL volume.

(iv) Sterile disposable tissue culture flasks with a flat growth area of approximately 25 or 75  $\text{cm}^2$ .

(v) Sterile disposable multiple well plates (24 wells)

(vi) Inverted microscope and stereomicroscope

(vii) Humidified incubator—maintain the conditions as follows: temperature,  $37^\circ\text{C}$ ;  $\text{CO}_2$  gas concentration, 5%.

#### 1.7.5. Control materials and substances

(i) Negative control material: polyethylene film

(ii) Positive control material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive control material (B): polyurethane film containing 0.25% zinc dibutyldithiocarbamate

(iv) Control substances: zinc diethyldithiocarbamate (reagent grade) and zinc dibutyldithiocarbamate (reagent grade)

#### 1.7.6. Test procedure

(i) 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  $2 \times 15\ \text{mm}$  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  $2.5\ \text{cm}^2$  and subject the pieces to the test without subdividing them into smaller pieces.

(ii) 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  $121^\circ\text{C}$  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 10 mL to one gram or

1 mL to  $2.5\ \text{cm}^2$  (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.

(iii) 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 – 1000 revolutions per minute for 2 – 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.

(iv) Cytotoxicity testing: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL 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 – 6 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL 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 – 9 days for L929 cells; 6 – 7 days for V79 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 TS from each well and add an appropriate volume of dilute Giemsa's TS 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 % extract concentration which inhibits colony formation to 50%,  $\text{IC}_{50}$  (%), 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.

## 2. Requirements for Plastic Containers for Aqueous Injections

### 2.1. 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 “1.4.1. Method 1”. When “1.4.1. Method 1” can not be applied, test according to “1.4.2.2. (ii) Method 2B”. In this case, the rate that the water-containing container is judged as “being turbid” is not more than 20%, and the rate that the reference suspension-containing container is judged as “being turbid” is not less than 80%.

(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 “1.5.1. Method 1”. The loss of mass is not more than 0.20%.

(4) Heavy metals <1.07>—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 “1.1.3.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(6) Cadmium—Perform the test as directed in “1.1.4.1. Method 1”. The absorbance of the sample solution is not more than that of the standard solution.

(7) Residue on ignition <2.44>—Not more than 0.1% (5 g).

(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) Residue on evaporation: Not more than 1.0 mg.

(9) Cytotoxicity—IC<sub>50</sub> (%) is not less than 90%. The result obtained by the other standard methods is negative.

### 2.2. Polyvinyl chloride containers for aqueous injections

The containers are composed of homopolymer of vinyl chloride, free from any adhesive, and the plasticizer added to the material should be di(2-ethylhexyl)phthalate. The containers may be covered with easily removable material to prevent the permeation of water vapor. In this case, perform the water vapor permeability test on the covered containers.

(1) Thickness—Measure the thickness of a container at five different locations. The difference between the maximum and minimum values of thickness is 0.05 mm or less.

(2) Transparency—Proceed as directed in (1) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(3) Appearance—Proceed as directed in (2) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(4) Leakage—Proceed with the test according to “1.6.

Leakage test”. The solution contained does not leak.

(5) Flexibility—Insert the spike needle for infusion through a rubber closure of the container used in (4) Leakage. The contained solution is almost completely discharged without displacement by air.

(6) Water vapor permeability—Proceed as directed in (3) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(7) Heavy metals <1.07>—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.

(8) Lead—Perform the test as directed in “1.1.3.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(9) Cadmium—Perform the test as directed in “1.1.4.2. Method 2”. The absorbance of the sample solution is not more than that of the standard solution.

(10) Tin—The absorbance of the sample solution is not more than that of the standard solution.

(11) Vinyl chloride—Wash cut pieces of a container with water, wipe them thoroughly with a filter paper, subdivide them into pieces smaller than 5 mm square, and put 0.5 g of them into a 20-mL vial. Add 2.5 mL of *N,N*-dimethylacetamide to the vial to dissolve the sample pieces, put a tight stopper on the vial, and use the solution in the vial as the sample solution. If the sample is hardly soluble, allow to stand the vial at room temperature for a night, put a tight stopper on the vial, and use the liquid part in the vial as the sample solution. Separately, to a 20-mL vial add 2.5 mL of *N,N*-dimethylacetamide, add 50  $\mu$ L of Standard Vinyl Chloride Solution, previously cooled with dry ice-methanol, put a tight stopper on the vial, and use the solution in the vial as the standard solution.

After heating the vials containing sample solution and standard solution at 90°C for 1 hour, perform the test with 0.5 mL each of vapor phases in these vials as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of vinyl chloride obtained from the sample solution is not larger than that from the standard solution.

*Operating conditions—*

Detector: A hydrogen flame-ionization detector.

Column: A fused silicate column 0.25 mm in inside diameter and 25 m in length, coated the inside surface in 3  $\mu$ m thickness with porous styrene-divinylbenzene copolymer for gas chromatography.

Column temperature: Maintain at 50°C for 2 minutes after injection, then rise to 120°C in the rate of 10°C per minute, then rise to 250°C in the rate of 20°C per minute, and keep at 250°C for 10 minutes.

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 7 minutes.

Split ratio: 1:5.

*System suitability—*

System performance: When the procedure is run under the above operating conditions with 0.5 mL of the vapor phase of the standard solution heated at 90°C for 1 hour, vinyl chloride and ethanol are eluted in this order with the resolu-

tion between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times under the above operating conditions with 0.5 mL of the vapor phase of the standard solution heated at 90°C for 1 hour, the relative standard deviation of the peak area of vinyl chloride is not more than 5.0%.

(12) Fine particles—The number of fine particles in 1.0 mL of the test solution is counted as not more than 100 of 5 to 10  $\mu\text{m}$ , not more than 10 of 10 to 25  $\mu\text{m}$  and not more than 1 of 25  $\mu\text{m}$  or more.

(13) Residue on ignition <2.44>—Not more than 0.1% (5 g).

(14) Extractable substances—Proceed as directed in (8) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(15) Cytotoxicity—Proceed as directed in (9) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

### 2.3. 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 “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(2) Appearance—Proceed as directed in (2) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(3) Vapor permeability—Proceed as directed in (3) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

(4) Cytotoxicity—Proceed as directed in (9) under “2.1. Polyethylene or polypropylene containers for aqueous injections”.

## 7.03 Test for Rubber Closure for Aqueous Infusions

The rubber closure for aqueous infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL 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 minute 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-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric

acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20.0 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to 10.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—Air.

Lamp: Cadmium hollow-cathode lamp.

Wavelength: 228.8 nm.

### 2. Lead

To 1.0 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under (1), and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution obtained in (1) and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.

Supporting gas—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.

#### 3.1. 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%.

#### 3.2. Foam test

Place 5 mL 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.

### 3.3. pH <2.54>

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.

### 3.4. Zinc

To 10.0 mL of the test solution add diluted dilute nitric acid (1 in 3) to make 20 mL, and use this solution as the sample 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 <2.23>, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene.

Supporting gas—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. One mL of this solution contains 0.01 mg of zinc (Zn).

### 3.5. Potassium Permanganate-reducing substances

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 <2.50> 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 in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

### 3.6. 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.

### 3.7. UV spectrum

Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.54>: it is not more than 0.20.

## 4. Acute systemic toxicity

The test solution meets the requirements, when examined under the following conditions against the blank solution.

### 4.1. Preparation of the test solution and the blank solution

Wash the rubber closures with water and Water 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, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the test solution. The blank solution is prepared in the same manner.

### 4.2. Test procedures

(i) Test animals: Use healthy male mice of inbred strain or from a closed colony, weighing 17 to 23 g.

(ii) Procedure: Separate the animals into two groups of 10 mice, and inject intravenously 50 mL each of the solutions per kg body mass.

### 4.3. 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 4.1. meets the requirements of the Pyrogen Test <4.04> as does the blank solution.

## 6. Hemolysis test

When 0.1 mL of defibrinated blood of rabbit is added to 10 mL of the test solution specified in 4.1. 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 10 mL of the blank solution.

## 8. Other Methods

### 8.01 Sterilization and Aseptic Manipulation

#### 1. Sterilization

Sterilization means a process whereby the killing or removal of all living microorganisms is accomplished. Generally, the sterilization process requires the choice of appropriate procedure and accurately controlled operation and conditions depending on the kind of microorganism, the conditions of contamination and the quality and nature of the substance to be sterilized.

The adequacy of sterilization is decided by means of the Sterility Test <4.06>.

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.

#### 2. Aseptic manipulation

Aseptic manipulation is a technique used for processing the sterile drug products which are not terminally sterilized in their final containers, and applied to a series of aseptic processing of the sterile products which are prepared by the filtration sterilization and/or with sterile raw materials.

Generally, aseptic manipulation requires the presterilization of all equipments and materials used for processing the 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 maintained.

## 9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.

### Reference Standards

#### 9.01 Reference Standards

Reference Standards are the reference substances prepared to a specified quality necessary with regard to their intended use as prescribed in monographs of the Pharmacopoeia.

The Japanese Pharmacopoeia Reference Standards are as follows:

(1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately.

- Aceglutamide RS
- Acetaminophen RS
- Aciclovir RS
- Adrenaline Bitartrate RS
- Alendronate Sodium RS
- Alprostadil RS
- p*-Aminobenzoyl Glutamic Acid RS
- Amitriptyline Hydrochloride RS
- Amlexanox RS
- Amlodipine Besilate RS
- Anhydrous Lactose RS
- Ascorbic Acid RS
- Aspirin RS
- Atorvastatin Calcium RS
- Atropine Sulfate RS
- Azathioprine RS
- Baclofen RS
- Baicalin RS
- Beclometasone Dipropionate RS
- Berberine Chloride RS
- Betamethasone RS
- Betamethasone Sodium Phosphate RS
- Betamethasone Valerate RS
- Bisacodyl RS
- Caffeine RS
- Calcitonin (Salmon) RS
- Calcium Folate RS
- Calcium Oxalate Monohydrate RS
- Camostat Mesilate RS
- d*-Camphor RS
- dl*-Camphor RS
- Carbidopa RS
- Cellulose RS
- Chlordiazepoxide RS
- Chlormadinone Acetate RS
- Chlorpheniramine Maleate RS
- Cholecalciferol RS
- Ciclosporin RS
- Cilostazol RS
- Cisplatin RS
- Clobetasol Propionate RS
- Clofibrate RS
- Clomifene Citrate RS
- Cortisone Acetate RS
- Cyanocobalamin RS
- Danazol RS
- Deferoxamine Mesilate RS
- Deslanoside RS
- Dexamethasone RS
- Diclofenamide RS
- Diethylcarbamazine Citrate RS
- Diflucortolone Valerate RS
- Digitoxin RS
- Digoxin RS
- Dihydroergotoxine Mesilate RS
- Dobutamine Hydrochloride RS
- Donepezil Hydrochloride RS
- Doxazosin Mesilate RS
- Edrophonium Chloride RS
- Elcatonin RS
- Enalapril Maleate RS
- Endotoxin RS
- Epitioanol RS
- Ergocalciferol RS
- Ergometrine Maleate RS
- Estradiol Benzoate RS
- Estriol RS
- Ethenzamide RS
- Ethinylestradiol RS
- Ethyl Aminobenzoate RS
- Ethyl Icosapentate RS
- Etoposide RS
- Fexofenadine Hydrochloride RS
- Fludrocortisone Acetate RS
- Fluocinolone Acetonide RS
- Fluocinonide RS
- Fluorometholone RS
- Fluoxymesterone RS
- Flutamide RS
- Fluvoxamine Maleate RS
- Folic Acid RS
- Furosemide RS
- Fursultiamine Hydrochloride RS
- Gabexate Mesilate RS
- Gefarnate RS
- Ginsenoside R<sub>b1</sub> RS
- Ginsenoside R<sub>g1</sub> RS
- Gitoxin RS
- Glimepiride RS
- D-Glucuronolactone RS
- Glycyrrhizic Acid RS
- Gonadorelin Acetate RS
- Guafenesin RS
- Heparin Sodium RS
- Heparin Sodium for Physical and Chemical Test RS
- High-molecular Mass Urokinase RS
- Human Chorionic Gonadotrophin RS
- Human Insulin RS
- Human Menopausal Gonadotrophin RS
- Hydrochlorothiazide RS
- Hydrocortisone RS
- Hydrocortisone Acetate RS

Hydrocortisone Sodium Phosphate RS  
 Hydrocortisone Succinate RS  
 Idoxuridine RS  
 Imipramine Hydrochloride RS  
 Indapamide RS  
 Indomethacin RS  
 Interleukin-2 RS  
 Ipriflavone RS  
 Isoflurane RS  
 Kallidinogenase RS  
 Lactose RS  
 Lactulose RS  
 Lanatoside C RS  
 Limaprost RS  
 Losartan Potassium RS  
 Low-molecular Mass Heparin RS  
 Loxoprofen RS  
 Lysozyme RS  
 Maltose RS  
 Manidipine Hydrochloride RS  
 Mecobalamin RS  
 Melting Point Standard-Acetanilide RS  
 Melting Point Standard-Acetophenetidine RS  
 Melting Point Standard-Caffeine RS  
 Melting Point Standard-Sulfanilamide RS  
 Melting Point Standard-Sulfapyridine RS  
 Melting Point Standard-Vanillin RS  
 Menatetrenone RS  
 Mestranol RS  
 Methotrexate RS  
 Methoxsalen RS  
 Methyldopa RS  
 Methylergometrine Maleate RS  
 Methylprednisolone Succinate RS  
 Methyltestosterone RS  
 Metildigoxin RS  
 Mexiletine Hydrochloride RS  
 Mizoribine RS  
 Nabumetone RS  
 Nateglinide RS  
 Neostigmine Methylsulfate RS  
 Nicotinamide RS  
 Nicotinic Acid RS  
 Nilvadipine RS  
 Nizatidine RS  
 Noradrenaline Bitartrate RS  
 Norgestrel RS  
 Over-sulfated Chondroitin Sulfate RS  
 Oxytocin RS  
 Ozagrel Sodium RS  
 Paeoniflorin RS  
 Pentobarbital RS  
 Pemirolast Potassium RS  
 Perphenazine RS  
 Phytonadione RS  
 Pioglitazone Hydrochloride RS  
 Potassium Sucrose Octasulfate RS  
 Povidone RS  
 Pravastatin 1,1,3,3-tetramethylbutylammonium RS  
 Prazosin Hydrochloride RS  
 Prednisolone RS  
 Prednisolone Acetate RS  
 Prednisolone Succinate RS

Primidone RS  
 Probenecid RS  
 Probucof RS  
 Prochlorperazine Maleate RS  
 Progesterone RS  
 Propiverine Hydrochloride RS  
 Protamine Sulfate RS  
 Puerarin RS  
 Pyridoxine Hydrochloride RS  
 Rabepazole Sodium RS  
 Ranitidine Hydrochloride RS  
 Reserpine RS  
 Retinol Acetate RS  
 Retinol Palmitate RS  
 Riboflavin RS  
 Risedronic Acid RS  
 Ritodrine Hydrochloride RS  
 Roxatidine Acetate Hydrochloride RS  
 Saccharated Pepsin RS  
 Sarpogrelate Hydrochloride RS  
 Scopolamine Hydrobromide RS  
 Sennoside A RS  
 Sennoside B RS  
 Serum Gonadotrophin RS  
 Sevoflurane RS  
 Simvastatin RS  
 Spironolactone RS  
 Sulfadiazine Silver RS  
 Swertiamarin RS  
 Tacrolimus RS  
 Teprenone RS  
 Testosterone Propionate RS  
 Thiamine Chloride Hydrochloride RS  
 Thiamylal RS  
 Thrombin RS  
 Tocopherol RS  
 Tocopherol Acetate RS  
 Tocopherol Nicotinate RS  
 Tocopherol Succinate RS  
 Tolazamide RS  
 Tolbutamide RS  
 Tolnaftate RS  
 Tosufloxacin Tosilate RS  
 Tranexamic Acid RS  
 Trehalose RS  
 Triamcinolone RS  
 Triamcinolone Acetonide RS  
 Trichlormethiazide RS  
 Trihexyphenidyl Hydrochloride RS  
 Troxipide RS  
 Tyrosine RS  
 Ubidecarenone RS  
 Ulinastatin RS  
 Vasopressin RS  
 Vinblastine Sulfate RS  
 Vincristine Sulfate RS  
 Warfarin Potassium RS  
 Zidovudine RS

(2) The reference standards which are prepared by National Institute of Infectious Diseases.

Aclarubicin RS

Actinomycin D RS  
Amikacin Sulfate RS  
Amoxicillin RS  
Amphotericin B RS  
Ampicillin RS  
Arbekacin Sulfate RS  
Aspoxicillin RS  
Azithromycin RS  
Aztreonam RS  
Bacampicillin Hydrochloride RS  
Bacitracin RS  
Bekanamycin Sulfate RS  
Benzylpenicillin Potassium RS  
Bleomycin A<sub>2</sub> Hydrochloride RS  
Carumonam Sodium RS  
Cefaclor RS  
Cefadroxil RS  
Cefalexin RS  
Cefalotin Sodium RS  
Cefatrizine Propylene Glycolate RS  
Cefazolin RS  
Cefbuperazone RS  
Cefcapene Pivoxil Hydrochloride RS  
Cefdinir RS  
Cefditoren Pivoxil RS  
Cefepime Dihydrochloride RS  
Cefixime RS  
Cefmenoxime Hydrochloride RS  
Cefmetazole RS  
Cefminox Sodium RS  
Cefodizime Sodium RS  
Cefoperazone RS  
Cefotaxime RS  
Cefotetan RS  
Cefotiam Hexetil Hydrochloride RS  
Cefotiam Hydrochloride RS  
Cefozopran Hydrochloride RS  
Cefpiramide RS  
Cefpirome Sulfate RS  
Cefpodoxime Proxetil RS  
Cefroxadine RS  
Cefsulodin Sodium RS  
Ceftazidime RS  
Cefteram Pivoxil Mesitylene Sulfonate RS  
Ceftibuten Hydrochloride RS  
Ceftizoxime RS  
Ceftriaxone Sodium RS  
Cefuroxime Axetil RS  
Chloramphenicol RS  
Chloramphenicol Palmitate RS  
Chloramphenicol Succinate RS  
Ciclacillin RS  
Clarithromycin RS  
Clindamycin Hydrochloride RS  
Clindamycin Phosphate RS  
Cloxacillin Sodium RS  
Colistin Sodium Methanesulfonate RS  
Colistin Sulfate RS  
Cycloserine RS  
Daunorubicin Hydrochloride RS  
Demethylchlortetracycline Hydrochloride RS  
Dibekacin Sulfate RS  
Dicloxacillin Sodium RS  
Diethanolammonium Fusidate RS  
Doxorubicin Hydrochloride RS  
Doxycycline Hydrochloride RS  
Enviomycin Sulfate RS  
Epirubicin Hydrochloride RS  
Erythromycin RS  
Faropenem Sodium RS  
Flomoxef Triethylammonium RS  
Fosfomycin Phenethylammonium RS  
Fradimycin Sulfate RS  
Gentamicin Sulfate RS  
Gramicidin RS  
Griseofulvin RS  
Idarubicin Hydrochloride RS  
Imipenem RS  
Isepamicin Sulfate RS  
Josamycin RS  
Josamycin Propionate RS  
Kanamycin Monosulfate RS  
Latamoxef Ammonium RS  
Lenampicillin Hydrochloride RS  
Leucomycin A<sub>5</sub> RS  
Lincomycin Hydrochloride RS  
Lithium Clavulanate RS  
Meropenem RS  
Micronomicin Sulfate RS  
Midecamycin RS  
Midecamycin Acetate RS  
Minocycline Hydrochloride RS  
Mitomycin C RS  
Mupirocin Lithium RS  
Nystatin RS  
Oxytetracycline Hydrochloride RS  
Panipenem RS  
Peplomycin Sulfate RS  
Phenethicillin Potassium RS  
Pimaricin RS  
Piperacillin RS  
Pirarubicin RS  
Pivmecillinam Hydrochloride RS  
Polymixin B Sulfate RS  
Pyrrolnitrin RS  
Ribostamycin Sulfate RS  
Rifampicin RS  
Rokitamycin RS  
Roxithromycin RS  
Siccanin RS  
Spectinomycin Hydrochloride RS  
Spiramycin Acetate II RS  
Streptomycin Sulfate RS  
Sulbactam RS  
Sulbenicillin Sodium RS  
Sultamicillin Tosilate RS  
Talampicillin Hydrochloride RS  
Tazobactam RS  
Teicoplanin RS  
Tetracycline Hydrochloride RS  
Tobramycin RS  
Trichomycin RS  
Vancomycin Hydrochloride RS  
Zinostatin Stimalamer RS

## Standard Solutions

### 9.21 Standard Solutions for Volumetric Analysis

Standard Solutions for Volumetric Analysis are the solutions of reagent with an accurately known concentration, mainly used for the volumetric analysis. They are prepared to a specified molar concentration. A 1 molar solution is a solution which contains exactly 1 mole of a specified substance in each 1000 mL of the solution and is designated as 1 mol/L. If necessary, these solutions are diluted to other specified molar concentrations and the diluted solutions are also 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 directed, standard solutions for volumetric analysis should be stored in colorless or light-resistant, glass-stoppered bottles.

#### Preparation and Standardization

A volumetric standard solution is prepared according to one of the following methods. The degree of difference from a specified concentration  $n$  (mol/L) is expressed as a factor (molar concentration coefficient)  $f$ . Usually, standard solutions are prepared so that the factor is in the range of 0.970 – 1.030. The determination procedure of the factor 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 1000 mL to prepare a standard solution having a concentration close to the specified molarity  $n$  (mol/L). In this case, the factor  $f$  of the standard solution is obtained by dividing the mass of the pure substance taken (g) by the molecular mass of the substance (g) and the specified molarity number  $n$ .

When a pure substance is not obtainable, it is permissible to use a highly purified substance whose purity has been exactly determined and certified.

(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 1000 mL to prepare a standard solution having a concentration close to the specified molarity  $n$  (mol/L). The factor  $f$  of this solution is determined by applying the standardization procedure described for the respective standard solution. The procedure is classified into direct and indirect methods, as follows:

#### 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  $f$  by applying the following equation.

$$f = \frac{1000m}{VMn}$$

$M$ : 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)

$n$ : Arithmetical mole number of the specified molar concentration of the standard solution to be 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.

$$f_2 = \frac{V_1 \times f_1}{V_2}$$

$f_1$ : Factor of the titrating standard solution having a known factor

$f_2$ : Factor of the prepared standard solution to be standardized

$V_1$ : Volume of the titrating standard solution consumed (mL)

$V_2$ : Volume of the prepared standard solution taken (mL)

(3) Standard solutions may be 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 to remain constant.

#### Ammonium Iron (II) Sulfate, 0.1 mol/L

1000 mL of this solution contains 39.214 g of ammonium iron (II) sulfate hexahydrate  $[\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$ : 392.14].

*Preparation*—Dissolve 40 g of ammonium iron (II) sulfate hexahydrate in a cooled mixture of 30 mL of sulfuric acid and 300 mL of water, dilute with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared ammonium iron (II) sulfate solution, and add 25 mL of water and 5 mL of phosphoric acid. Titrate <2.50> 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

1000 mL of this solution contains 7.843 g of ammonium iron (II) sulfate hexahydrate  $[\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$ : 392.14].

*Preparation*—Before 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

1000 mL of this solution contains 48.22 g of ammonium iron (III) sulfate dodecahydrate  $[\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$ : 482.19].

*Preparation*—Dissolve 49 g of ammonium iron (III) sulfate dodecahydrate in a cooled mixture of 6 mL of sulfuric acid and 300 mL of water, add water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared ammonium iron (III) sulfate solution into an iodine flask, add 5 mL of hydrochloric acid, and shake the mixture. Dis-

solve 2 g of potassium iodide, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 50 mL of water, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

#### Ammonium Thiocyanate, 0.1 mol/L

1000 mL of this solution contains 7.612 g of ammonium thiocyanate ( $\text{NH}_4\text{SCN}$ : 76.12).

*Preparation*—Dissolve 8 g of ammonium thiocyanate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the 0.1 mol/L silver nitrate VS, and add 50 mL of water, 2 mL of nitric acid and 2 mL of ammonium iron (III) sulfate TS. Titrate <2.50> 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

1000 mL of this solution contains 1.5224 g of ammonium thiocyanate ( $\text{NH}_4\text{SCN}$ : 76.12).

*Preparation*—Before use, dilute 0.1 mol/L ammonium thiocyanate VS with water to make exactly 5 times the initial volume.

#### Barium chloride, 0.1 mol/L

1000 mL of this solution contains 24.426 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

*Preparation*—Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 20 mL of the prepared solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter 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 ( $\text{BaSO}_4$ ).

Each mL of 0.1 mol/L barium chloride VS  
= 23.34 mg of  $\text{BaSO}_4$

#### Barium Chloride, 0.02 mol/L

1000 mL of this solution contains 4.885 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

*Preparation*—Dissolve 4.9 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 100 mL of the prepared barium chloride solution, add 3 mL of hydrochloric acid,

and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), warmed previously, heat the mixture on a water bath for 30 minutes, and allow to stand overnight. Filter the mixture, wash the collected precipitate of 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 strongly again at about 700°C for 2 hours. After cooling, weigh accurately the residue as barium sulfate ( $\text{BaSO}_4$ ), and calculate the molarity factor.

Each mL of 0.02 mol/L barium chloride VS  
= 4.668 mg of  $\text{BaSO}_4$

#### Barium Chloride, 0.01 mol/L

1000 mL of this solution contains 2.4426 g of barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ : 244.26).

*Preparation*—Before use, dilute 0.02 mol/L barium chloride VS with water to make exactly twice the initial volume.

#### Barium Perchlorate, 0.005 mol/L

1000 mL of this solution contains 1.6812 g of barium perchlorate [ $\text{Ba}(\text{ClO}_4)_2$ : 336.23].

*Preparation*—Dissolve 1.7 g of barium perchlorate in 200 mL of water, dilute with 2-propanol to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 20 mL of the prepared barium perchlorate solution, add 55 mL of methanol and 0.15 mL of arsenazo III TS. Titrate <2.50> the solution with 0.005 mol/L sulfuric acid VS until its purple color changes through red-purple to red. Calculate the molarity factor.

#### Bismuth Nitrate, 0.01 mol/L

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate [ $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ : 485.07].

*Preparation*—Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS, and titrate <2.50> the solution with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red color changes to yellow. Calculate the molarity factor.

#### Bromine, 0.05 mol/L

1000 mL 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 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared solution into an iodine flask. Add 120 mL of water, quickly add 5 mL of hydrochloric acid, stopper the flask immediately, and shake it gently. Then add 5 mL of potassium iodide TS, re-stopper immediately, shake the mixture gently, and allow to stand for 5 minutes. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform 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 (IV) Tetraammonium Sulfate, 0.1 mol/L**

1000 mL of this solution contains 63.26 g of cerium (IV) tetraammonium sulfate dihydrate [Ce(NH<sub>4</sub>)<sub>4</sub>(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O: 632.55].

*Preparation*—Dissolve 64 g of cerium (VI) tetraammonium sulfate dihydrate in 0.5 mol/L sulfuric acid VS to make 1000 mL, allow to stand for 24 hours, filter the solution through a glass filter (G3 or G4), if necessary, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared cerium (IV) tetraammonium sulfate solution into an iodine flask. Add 20 mL of water and 20 mL of dilute sulfuric acid, then dissolve 1 g of potassium iodide in the mixture. Immediately titrate <2.50> the solution with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

**Cerium (IV) Tetraammonium Sulfate, 0.01 mol/L**

1000 mL of this solution contains 6.326 g of cerium (IV) tetraammonium sulfate dihydrate [Ce(NH<sub>4</sub>)<sub>4</sub>(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O: 632.55].

*Preparation*—Before 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 Dihydrogen Ethylenediamine Tetraacetate, 0.1 mol/L**

1000 mL of this solution contains 37.224 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

*Preparation*—Dissolve 38 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Wash zinc (standard reagent) with dilute 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 this zinc, add 20 mL of dilute hydrochloric acid and 8 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Pipet 25 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 6.538 mg of Zn

Note: Store in polyethylene bottles.

**Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.05 mol/L**

1000 mL of this solution contains 18.612 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

*Preparation*—Dissolve 19 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Wash zinc (standard reagent) with dilute 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 0.8 g of this zinc, add 12 mL of dilute 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 200 mL. Measure exactly 20 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 3.269 mg of Zn

Note: Store in polyethylene bottles.

**Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.02 mol/L**

1000 mL of this solution contains 7.445 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

*Preparation*—Dissolve 7.5 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, but weigh accurately 0.3 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and with acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, and add 5 mL of dilute hydrochloric acid and 5 drops of bromine TS.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 1.308 mg of Zn

Note: Store in polyethylene bottles.

**Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.01 mol/L**

1000 mL of this solution contains 3.7224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

*Preparation*—Before use, dilute 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly twice the initial volume.

**Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.001 mol/L**

1000 mL of this solution contains 0.37224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>·2H<sub>2</sub>O: 372.24).

*Preparation*—Before use, dilute 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate 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**

1000 mL of this solution contains 72.92 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 180 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh about 1.5 g of sodium carbonate (standard reagent) accurately, and dissolve in 100 mL of water.

Each mL of 2 mol/L hydrochloric acid VS  
= 106.0 mg of  $\text{Na}_2\text{CO}_3$

**Hydrochloric Acid, 1 mol/L**

1000 mL of this solution contains 36.461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 90 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.8 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 50 mL of water, and titrate <2.50> with the prepared hydrochloric acid to calculate the molarity factor (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end-point is approached, boil the content 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. In the potentiometric titration, titrate with vigorous stirring, without boiling.

Each mL of 1 mol/L hydrochloric acid VS  
= 53.00 mg of  $\text{Na}_2\text{CO}_3$

**Hydrochloric Acid, 0.5 mol/L**

1000 mL of this solution contains 18.230 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 45 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.5 mol/L hydrochloric acid VS  
= 26.50 mg of  $\text{Na}_2\text{CO}_3$

**Hydrochloric Acid, 0.2 mol/L**

1000 mL of this solution contains 7.292 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Dilute 18 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.2 mol/L hydrochloric acid VS  
= 10.60 mg of  $\text{Na}_2\text{CO}_3$

**Hydrochloric Acid, 0.1 mol/L**

1000 mL of this solution contains 3.6461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly twice the initial volume.

Each mL of 0.1 mol/L hydrochloric acid VS  
= 5.300 mg of  $\text{Na}_2\text{CO}_3$

**Hydrochloric Acid, 0.05 mol/L**

1000 mL of this solution contains 1.8230 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 4 times the initial volume.

**Hydrochloric Acid, 0.02 mol/L**

1000 mL of this solution contains 0.7292 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 10 times the initial volume.

**Hydrochloric Acid, 0.01 mol/L**

1000 mL of this solution contains 0.36461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 20 times the initial volume.

**Hydrochloric Acid, 0.001 mol/L**

1000 mL of this solution contains 0.036461 g of hydrochloric acid (HCl: 36.46).

*Preparation*—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 200 times the initial volume.

**Iodine, 0.05 mol/L**

1000 mL of this solution contains 12.690 g of iodine (I: 126.90).

*Preparation*—Dissolve 13 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid and water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 15 mL of the iodine solution, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (Indicator method: starch TS; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS, and continue the titration

until the blue color disappears. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized before use.

**Iodine, 0.01 mol/L**

1000 mL of this solution contains 2.5381 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 5 times the initial volume.

**Iodine, 0.005 mol/L**

1000 mL of this solution contains 1.2690 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 10 times the initial volume.

**Iodine, 0.002 mol/L**

1000 mL of this solution contains 0.5076 g of iodine (I: 126.90).

*Preparation*—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 25 times the initial volume.

**Magnesium Chloride, 0.05 mol/L**

1000 mL of this solution contains 10.165 g of magnesium chloride hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ : 203.30).

*Preparation*—Dissolve 10.2 g of magnesium chloride hexahydrate in freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared magnesium chloride solution. Add 50 mL of water, 3 mL of pH 10.7 ammonia-ammonium chloride buffer solution and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

**Magnesium Chloride, 0.01 mol/L**

1000 mL of this solution contains 2.0330 g of magnesium chloride hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ : 203.30).

*Preparation*—Before use, dilute 0.05 mol/L magnesium chloride VS with water to make exactly 5 times the initial volume.

**Oxalic Acid, 0.05 mol/L**

1000 mL of this solution contains 6.303 g of oxalic acid ( $\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ : 126.07).

*Preparation*—Dissolve 6.3 g of oxalic acid dihydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared oxalic acid solution in a 500-mL conical flask, and add 200 mL of diluted sulfuric acid (1 in 20), previously boiled for 10 to 15 minutes and then cooled to  $27 \pm 3^\circ\text{C}$ . Transfer freshly standardized 0.02 mol/L potassium permanganate VS to a burette. Add quickly 22 mL of the 0.02 mol/L potassium permanganate VS to the oxalic acid solution from the burette under gentle stirring, and allow to stand until the red color of the mixture disappears. Heat the solution up to between  $55^\circ\text{C}$  and  $60^\circ\text{C}$ , and complete the titration <2.50> by adding 0.02 mol/L potassium permanganate VS until a faint red color persists for 30 seconds. Add the last 0.5 to 1 mL

dropwise, being particularly careful to allow the solution to become decolorized before the next drop is added. Calculate the molarity factor.

Note: Store protected from light.

**Oxalic Acid, 0.005 mol/L**

1000 mL of this solution contains 0.6303 g of oxalic acid dihydrate ( $\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ : 126.07).

*Preparation*—Before 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**

1000 mL of this solution contains 10.046 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

*Preparation*—Add slowly 8.7 mL of perchloric acid to 1000 mL of acetic acid (100) while keeping the temperature at about  $20^\circ\text{C}$ . Allow the mixture to stand for about 1 hour. Perform quickly the test as directed under Water Determination with 3.0 mL of the mixture, and designate the water content as A (g/dL). To the rest mixture add slowly  $[(A - 0.03) \times 52.2]$  mL of acetic anhydride with shaking at about  $20^\circ\text{C}$ . Allow the solution to stand for 24 hours, and standardize it as follows:

*Standardization*—Weigh accurately about 0.3 g of potassium hydrogen phthalate (standard reagent), previously dried at  $105^\circ\text{C}$  for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of acetic acid (100), and titrate <2.50> the solution with the prepared perchloric acid solution (Indicator method: 3 drops of crystal violet TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a blue color. Perform a blank determination. Calculate the molarity factor.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 20.42 \text{ mg of } \text{KHC}_8\text{H}_4(\text{COO})_2 \end{aligned}$$

Note: Store protected from moisture.

**Perchloric Acid, 0.05 mol/L**

1000 mL of this solution contains 5.023 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

*Preparation*—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly twice the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add  $[(A - 0.03) \times 52.2]$  mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

**Perchloric Acid, 0.02 mol/L**

1000 mL of this solution contains 2.0092 g of perchloric acid ( $\text{HClO}_4$ : 100.46).

*Preparation*—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly 5 times the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add  $[(A - 0.03) \times 52.2]$  mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

**Perchloric Acid-1,4-Dioxane, 0.1 mol/L**

1000 mL of this solution contains 10.046 g of perchloric

acid (HClO<sub>4</sub>: 100.46).

*Preparation*—Dilute 8.5 mL of perchloric acid with 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.5 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 80 mL of acetic acid for nonaqueous titration, and add 3 drops of crystal violet TS. Titrate <2.50> the solution with the prepared perchloric acid-1,4-dioxane solution until it acquires a blue color. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS  
= 20.42 mg of KHC<sub>8</sub>H<sub>4</sub>(COO)<sub>2</sub>

Note: Store in a cold place, protected from moisture.

#### Perchloric Acid-1,4-Dioxane, 0.05 mol/L

1000 mL of this solution contains 5.023 g of perchloric acid (HClO<sub>4</sub>: 100.46).

*Preparation*—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

#### Perchloric Acid-1,4-Dioxane, 0.004 mol/L

1000 mL of this solution contains 0.4018 g of perchloric acid (HClO<sub>4</sub>: 100.46).

*Preparation*—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly 25 times the initial volume.

#### Potassium Bichromate, 1/60 mol/L

See Potassium Dichromate, 1/60 mol/L.

#### Potassium Bromate, 1/60 mol/L

1000 mL of this solution contains 2.7833 g of potassium bromate (KBrO<sub>3</sub>: 167.00).

*Preparation*—Dissolve 2.8 g of potassium bromate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared potassium bromate solution into an iodine flask. Add 2 g of potassium iodide and 5 mL of dilute sulfuric acid, stopper the flask, and allow the solution to stand for 5 minutes. Add 100 mL of water, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

#### Potassium Dichromate, 1/60 mol/L

1000 mL of this solution contains 4.903 g of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>: 294.18).

*Preparation*—Weigh accurately about 4.903 g of potassium dichromate (standard reagent), previously powdered, dried between 100°C and 110°C for 3 to 4 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and 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

1000 mL of this solution contains 32.924 g of potassium hexacyanoferrate (III) [K<sub>3</sub>Fe(CN)<sub>6</sub>: 329.24].

*Preparation*—Dissolve 33 g of potassium hexacyanoferrate (III) in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 25 mL of the prepared potassium hexacyanoferrate (III) solution into an iodine flask. Add 2 g of potassium iodide and 10 mL of dilute hydrochloric acid, stopper the flask, and allow to stand for 15 minutes. Add 15 mL of zinc sulfate TS, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

#### Potassium Hexacyanoferrate (III), 0.05 mol/L

1000 mL of this solution contains 16.462 g of potassium hexacyanoferrate (III) [K<sub>3</sub>Fe(CN)<sub>6</sub>: 329.24].

*Preparation*—Before use, dilute 0.1 mol/L potassium hexacyanoferrate (III) VS with water to make exactly twice the initial volume.

#### Potassium Hydroxide, 1 mol/L

1000 mL of this solution contains 56.11 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Dissolve 65 g of potassium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Shake the mixture thoroughly, and 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 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 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate <2.50> the solution with the prepared potassium hydroxide solution until it acquires a green color. Calculate the molarity factor.

Each mL of 1 mol/L potassium hydroxide VS  
= 97.09 mg of HOSO<sub>2</sub>NH<sub>2</sub>

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.

#### Potassium Hydroxide, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 32 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 1.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L potassium hydroxide VS  
= 48.55 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

#### Potassium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 6.5 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 0.25 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L potassium hydroxide VS  
= 9.709 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

#### Potassium Hydroxide-Ethanol, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Dissolve 35 g of potassium hydroxide in 20 mL of water, and add aldehyde-free ethanol to make 1000 mL. Allow the solution to stand for 24 hours in a tightly stoppered bottle. Then quickly decant the supernatant liquid, and standardize the solution as follows:

*Standardization*—Measure exactly 15 mL of 0.25 mol/L sulfuric acid VS, add 50 mL of water, and titrate with the prepared potassium hydroxide-ethanol solution to calculate the molarity factor (Indicator method: 2 drops of phenolphthalein TS; or potentiometric titration). In the indicator method, titrate <2.50> until the solution acquires a pale red color.

Note: Store in tightly stoppered bottles, protected from light. Standardize before use.

#### Potassium Hydroxide-Ethanol, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

*Preparation*—Weigh 7 g of potassium hydroxide, proceed as directed for preparation under 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.5 mol/L potassium hydroxide-ethanol VS, but measure exactly 15 mL 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 Iodate, 0.05 mol/L

1000 mL of this solution contains 10.700 g of potassium iodate ( $\text{KIO}_3$ : 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 1000 mL. Calculate the molarity factor.

#### Potassium Iodate, 1/60 mol/L

1000 mL of this solution contains 3.567 g of potassium iodate ( $\text{KIO}_3$ : 214.00).

*Preparation*—Weigh accurately about 3.567 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

#### Potassium Iodate, 1/1200 mol/L

1000 mL of this solution contains 0.17833 g of potassium iodate ( $\text{KIO}_3$ : 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 1000 mL. Calculate the molarity factor.

#### Potassium Permanganate, 0.02 mol/L

1000 mL of this solution contains 3.1607 g of potassium permanganate ( $\text{KMnO}_4$ : 158.03).

*Preparation*—Dissolve 3.2 g of potassium permanganate in water to make 1000 mL, 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 500 mL conical flask, dissolve in 30 mL of water, add 250 mL 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 40 mL 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 <2.50> with the potassium permanganate solution until a faint red color persists for 30 seconds. Add the last 0.5 to 1 mL 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  $\text{Na}_2\text{C}_2\text{O}_4$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

#### Potassium Permanganate, 0.002 mol/L

1000 mL of this solution contains 0.31607 g of potassium permanganate ( $\text{KMnO}_4$ : 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

1000 mL of this solution contains 16.987 g of silver nitrate ( $\text{AgNO}_3$ : 169.87).

*Preparation*—Dissolve 17.0 g of silver nitrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 80 mg 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 50 mL of water, and titrate <2.50> under vigorous stirring with the prepared silver nitrate solution to calculate the molarity factor (Indicator method: 3 drops of fluorescein sodium TS; or potentiometric titration: silver electrode). In the indicator method, titrate until the color of the solution changes from yellow-green to orange through yellow.

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

1000 mL of this solution contains 3.3974 g of silver nitrate (AgNO<sub>3</sub>: 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

1000 mL of this solution contains 1.6987 g of silver nitrate (AgNO<sub>3</sub>: 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

1000 mL of this solution contains 0.8494 g of silver nitrate (AgNO<sub>3</sub>: 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, 0.001 mol/L

1000 mL of this solution contains 0.16987 g of silver nitrate (AgNO<sub>3</sub>: 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

1000 mL of this solution contains 8.203 g of sodium acetate (CH<sub>3</sub>COONa: 82.03).

*Preparation*—Dissolve 8.20 g of anhydrous sodium acetate in acetic acid (100) to make 1000 mL, and standardize the solution as follows:

*Standardization*—Pipet 25 mL of the prepared sodium acetate solution, add 50 mL of acetic acid (100) and 1 mL of *p*-naphtholbenzene TS, and titrate <2.50> 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

1000 mL of this solution contains 39.997 g of sodium hydroxide (NaOH: 40.00).

*Preparation*—Dissolve 42 g of sodium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate 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 1.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it

in 25 mL of freshly boiled and cooled water, and titrate <2.50> the solution with the prepared sodium hydroxide solution to calculate the molarity factor (Indicator method: 2 drops of bromothymol blue TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a green color.

Each mL of 1 mol/L sodium hydroxide VS  
= 97.09 mg of HOSO<sub>2</sub>NH<sub>2</sub>

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

1000 mL of this solution contains 19.999 g of sodium hydroxide (NaOH: 40.00).

*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 0.7 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L sodium hydroxide VS  
= 48.55 mg of HOSO<sub>2</sub>NH<sub>2</sub>

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

1000 mL of this solution contains 7.999 g of sodium hydroxide (NaOH: 40.00).

*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 0.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.2 mol/L sodium hydroxide VS  
= 19.42 mg of HOSO<sub>2</sub>NH<sub>2</sub>

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

1000 mL of this solution contains 3.9997 g of sodium hydroxide (NaOH: 40.00).

*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 0.15 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.709 mg of HOSO<sub>2</sub>NH<sub>2</sub>

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**

1000 mL of this solution contains 1.9999 g of sodium hydroxide (NaOH: 40.00).

*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**

1000 mL of this solution contains 0.7999 g of sodium hydroxide (NaOH: 40.00).

*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**

1000 mL of this solution contains 0.39997 g of sodium hydroxide (NaOH: 40.00).

*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 Hydroxide-Ethanol, 0.025 mol/L**

1000 mL of this solution contains 1.000 g of sodium hydroxide (NaOH: 40.00).

*Preparation*—Dissolve 2.1 g of sodium hydroxide in 100 mL of ethanol (99.5), stopper tightly, and allow to stand for a night. To 50 mL of the supernatant liquid add 650 mL of ethanol (99.5) and freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 25 mg of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for 48 hours. Dissolve in 30 mL of diluted ethanol (99.5) with freshly boiled and cooled water (7 in 10), and titrate <2.50> with the prepared sodium hydroxide-ethanol to calculate the molarity factor (potentiometric titration).

Each mL of 0.025 mol/L sodium hydroxide-ethanol VS  
= 2.427 mg of  $\text{HOSO}_2\text{NH}_2$

Note: Store in light-resistant, well-stoppered bottles. The standardization should be performed before using.

**Sodium Lauryl Sulfate, 0.01 mol/L**

1000 mL of this solution contains 2.8838 g of sodium lauryl sulfate ( $\text{C}_{12}\text{H}_{25}\text{NaO}_4\text{S}$ : 288.38).

*Preparation*—Dissolve 2.9 g of sodium lauryl sulfate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.3 g of papaverine hydrochloride for assay, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a glass-stoppered conical flask, add 5 mL each of water and dilute sulfuric acid and 60 mL of dichloromethane, then add 5 to 6 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.50>, while vigorous shaking, with the sodium lauryl sulfate solution prepared above, using a buret with a minimum graduation of 0.02 mL. End point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of the sodium lauryl sulfate solution, vigorous shaking and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS  
= 3.759 mg of  $\text{C}_{20}\text{H}_{21}\text{NO}_4\cdot\text{HCl}$

**Sodium Methoxide, 0.1 mol/L**

1000 mL of this solution contains 5.402 g of sodium methoxide ( $\text{CH}_3\text{ONa}$ : 54.02).

*Preparation*—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add benzene to make 1000 mL, 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 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide VS  
= 12.21 mg of  $\text{C}_6\text{H}_5\text{COOH}$

Note: Store in a cold place, protected from moisture. Standardize before use.

**Sodium Methoxide-Dioxane, 0.1 mol/L**

See Sodium Methoxide-1,4-Dioxane, 0.1 mol/L.

**Sodium Methoxide-1,4-Dioxane, 0.1 mol/L**

1000 mL of this solution contains 5.402 g of sodium methoxide ( $\text{CH}_3\text{ONa}$ : 54.02).

*Preparation*—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add 1,4-dioxane to make 1000 mL, 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 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide-1,4-dioxane solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide-1,4-dioxane VS  
= 12.21 mg of  $\text{C}_6\text{H}_5\text{COOH}$

Note: Store in a cold place, protected from moisture. Standardize before use.

**Sodium Nitrite, 0.1 mol/L**

1000 mL of this solution contains 6.900 g of sodium nitrite ( $\text{NaNO}_2$ : 69.00).

*Preparation*—Dissolve 7.2 g of sodium nitrite in water to make 1000 mL, 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 10 mL of hydrochloric acid, 40 mL of water and 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate with the prepared sodium nitrite solution as directed in the potentiometric titration or amperometric titration under Endpoint Detection Methods in Titrimetry <2.50>. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium nitrite VS  
= 17.22 mg of  $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

**Sodium Oxalate, 0.005 mol/L**

1000 mL of this solution contains 0.6700 g of sodium oxalate ( $\text{Na}_2\text{C}_2\text{O}_4$ : 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 1000 mL, and calculate the molarity factor.

**Sodium Tetrphenylborate, 0.02 mol/L**

1000 mL of this solution contains 6.844 g of sodium tetrphenylborate [ $\text{NaB}(\text{C}_6\text{H}_5)_4$ : 342.22].

*Preparation*—Dissolve 7.0 g of sodium tetrphenylborate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh 0.5 g of potassium hydrogen phthalate (standard reagent), dissolve it in 100 mL of water, add 2 mL of acetic acid (31), and warm to 50°C in a water bath. Add slowly 50 mL of the prepared sodium tetrphenylborate 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 5 mL portions of potassium tetrphenylborate TS, dry at 105°C for 1 hour, and weigh accurately the glass filter. Calculate the molarity factor from the mass of potassium tetrphenylborate [ $\text{KB}(\text{C}_6\text{H}_5)_4$ : 358.32].

Each mL of 0.02 mol/L sodium tetrphenylborate VS  
= 7.166 mg of  $\text{KB}(\text{C}_6\text{H}_5)_4$

Note: Prepare before use.

**Sodium Tetrphenylboron, 0.02 mol/L**

See Sodium Tetrphenylborate, 0.02 mol/L.

**Sodium Thiosulfate, 0.1 mol/L**

1000 mL of this solution contains 24.818 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*Preparation*—Dissolve 25 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL, allow to stand for 24 hours, and standardize the solution as follows:

*Standardization*—Weigh accurately about 50 mg 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 transfer to an iodine flask. Dissolve it in 25 mL of water, add 2 g of potassium iodide and 10 mL of dilute sulfuric acid, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 100 mL of water, and titrate <2.50> the liberated iodine with the prepared sodium thiosulfate solution (Indicator method; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 3.567 mg of  $\text{KIO}_3$

Note: This solution, if stored for a long period, should be restandardized.

**Sodium Thiosulfate, 0.05 mol/L**

1000 mL of this solution contains 12.409 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*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**

1000 mL of this solution contains 4.964 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*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**

1000 mL of this solution contains 2.4818 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*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**

1000 mL of this solution contains 1.2409 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*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.

**Sodium Thiosulfate, 0.002 mol/L**

1000 mL of this solution contains 0.4964 g of sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ : 248.18).

*Preparation*—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 50 times the initial volume.

**Sulfuric Acid, 0.5 mol/L**

1000 mL of this solution contains 49.04 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

*Preparation*—Add slowly, under stirring, 30 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.8 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 50 mL of water, and titrate <2.50> the solution with the prepared sulfuric acid (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, 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. In the potentiometric titration, titrate with vigorous stirring without boiling.

Each mL of 0.5 mol/L sulfuric acid VS  
= 53.00 mg of  $\text{Na}_2\text{CO}_3$

**Sulfuric Acid, 0.25 mol/L**

1000 mL of this solution contains 24.520 g of sulfuric acid ( $\text{H}_2\text{SO}_4$ : 98.08).

*Preparation*—Add slowly, under stirring, 15 mL of sulfuric acid to 1000 mL 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 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.25 mol/L sulfuric acid VS  
= 26.50 mg of Na<sub>2</sub>CO<sub>3</sub>

#### Sulfuric Acid, 0.1 mol/L

1000 mL of this solution contains 9.808 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Add slowly, under stirring, 6 mL of sulfuric acid to 1000 mL 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 50 mL of water.

Each mL of 0.1 mol/L sulfuric acid VS  
= 10.60 mg of Na<sub>2</sub>CO<sub>3</sub>

#### Sulfuric Acid, 0.05 mol/L

1000 mL of this solution contains 4.904 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Add slowly, under stirring, 3 mL of sulfuric acid to 1000 mL 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 80 mg of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.05 mol/L sulfuric acid VS  
= 5.300 mg of Na<sub>2</sub>CO<sub>3</sub>

#### Sulfuric Acid, 0.025 mol/L

1000 mL of this solution contains 2.4520 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 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

1000 mL of this solution contains 0.9808 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 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

1000 mL of this solution contains 0.4904 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 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

1000 mL of this solution contains 0.04904 g of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>: 98.08).

*Preparation*—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 100 times the initial volume.

#### Tetrabutylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 25.947 g of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Preparation*—Before use, dilute a volume of 10%

tetrabutylammonium hydroxide-methanol TS, equivalent to 26.0 g of tetrabutylammonium hydroxide, with 2-propanol to make 1000 mL, 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 50 mL of acetone, and titrate <2.50> the solution with the prepared tetrabutylammonium hydroxide solution (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS  
= 12.21 mg of C<sub>6</sub>H<sub>5</sub>COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.2 mol/L

1000 mL of this solution contains 18.231 g of tetramethylammonium hydroxide [(CH<sub>3</sub>)<sub>4</sub>NOH: 91.15].

*Preparation*—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 18.4 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Weigh accurately about 0.4 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 60 mL of *N,N*-dimethylformamide, and titrate <2.50> the solution with the prepared 0.2 mol/L tetramethylammonium hydroxide solution (Indicator method: 3 drops of thymol blue-*N,N*-dimethylformamide TS; or potentiometric titration). In the indicator method, titrate until a blue color is produced. Perform a blank determination in the same manner. Calculate the molarity factor.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS  
= 24.42 mg of C<sub>6</sub>H<sub>5</sub>COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide [(CH<sub>3</sub>)<sub>4</sub>NOH: 91.15].

*Preparation*—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.2 mol/L tetramethylammonium hydroxide VS. Weigh accurately about 0.2 g of benzoic acid and titrate <2.50>.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS  
= 12.21 mg of C<sub>6</sub>H<sub>5</sub>COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

#### Tetramethylammonium Hydroxide, 0.02 mol/L

1000 mL of this solution contains 1.8231 g of tetramethylammonium hydroxide [(CH<sub>3</sub>)<sub>4</sub>NOH: 91.15].

*Preparation*—Before use, dilute 0.1 mol/L tetramethylammonium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

**Tetramethylammonium Hydroxide-Methanol, 0.1 mol/L**

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide [(CH<sub>3</sub>)<sub>4</sub>NOH: 91.15].

*Preparation*—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with methanol to make 1000 mL, and standardize the solution as follows:

*Standardization*—Proceed as directed for standardization under 0.1 mol/L tetramethylammonium 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**

1000 mL of this solution contains 15.423 g of titanium (III) chloride (TiCl<sub>3</sub>: 154.23).

*Preparation*—Add 75 mL of hydrochloric acid to 75 mL of titanium (III) chloride (20), and dilute with freshly boiled and cooled water to make 1000 mL. 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, 500 mL conical flask. Passing carbon dioxide through the flask, dissolve it in 50 mL of freshly boiled and cooled water, and add 25 mL of diluted sulfuric acid (27 in 100). Rapidly add exactly 40 mL of 0.02 mol/L potassium permanganate VS to the mixture, while passing carbon dioxide through the flask. Titrate <2.50> with the prepared titanium (III) chloride solution until the calculated end point is approached, then add 5 g of ammonium thiocyanate immediately, and continue the titration with the prepared titanium (III) chloride solution 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**

1000 mL of this solution contains 6.538 g of zinc (Zn: 65.38).

*Preparation*—To 6.538 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 80 mL of dilute hydrochloric acid and 2.5 mL of bromine TS, dissolve by gentle warming, evaporate excess bromine by boiling, and add water to make exactly 1000 mL.

**Zinc Acetate, 0.05 mol/L**

1000 mL of this solution contains 10.975 g of zinc acetate dihydrate [Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O: 219.50].

*Preparation*—Dissolve 11.1 g of zinc acetate dihydrate in 40 mL of water and 4 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Measure exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride reagent. Titrate <2.50> 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<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O: 219.50].

*Preparation*—Dissolve 4.43 g of zinc acetate dihydrate in 20 mL of water and 2 mL of dilute acetic acid, add water to make 1000 mL, 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 dihydrogen ethylenediamine tetraacetate VS.

**Zinc Sulfate, 0.1 mol/L**

1000 mL of this solution contains 28.755 g of zinc sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O: 287.55).

*Preparation*—Dissolve 28.8 g of zinc sulfate heptahydrate in water to make 1000 mL, and standardize the solution as follows:

*Standardization*—Pipet 25 mL of the prepared zinc sulfate solution, add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue-purple. Calculate the molarity factor.

**Zinc sulfate, 0.02 mol/L**

1000 mL of this solution contains 5.7510 g of zinc sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O: 287.55).

*Preparation*—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly 5 times the initial volume.

## 9.22 Standard Solutions

Standard Solutions are used as the standard for the comparison in a text of the Pharmacopoeia.

**Borate pH Standard Solution** See pH Determination <2.54>.

**Calcium Hydroxide pH Standard Solution** See pH Determination <2.54>.

**Carbonate pH Standard Solution** See pH Determination <2.54>.

**Formadin stock suspension** To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after allowing to stand at 25 ± 3°C for 24 hours. Store in a glass container free from surface defects. Use within 2 months. Shake thoroughly before use.

**Oxalate pH Standard Solution** See pH Determination <2.54>.

**pH Standard Solution, Borate** See pH Determination <2.54>.

**pH Standard Solution, Calcium Hydroxide** See pH Determination <2.54>.

**pH Standard Solution, Carbonate** See pH Determination <2.54>.

**pH Standard Solution, Oxalate** See pH Determination

<2.54>.

**pH Standard Solution, Phosphate** See pH Determination <2.54>.

**pH Standard Solution, Phthalate** See pH Determination <2.54>.

**Phosphate pH Standard Solution** See pH Determination <2.54>.

**Phthalate pH Standard Solution** See pH Determination <2.54>.

**Standard Aluminum Solution for Atomic Absorption Spectrophotometry** To exactly 10 mL of Standard Aluminum Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.100 mg of aluminum (Al).

**Standard Aluminum Stock Solution** Weigh 1.0 g of aluminum, add 60 mL of diluted hydrochloric acid (1 in 2), dissolve by heating, cool, add water to make 1000 mL. Pipet 10 mL of this solution, add 30 mL of water and 5 mL of acetic acid-ammonium acetate buffer solution, pH 3.0, and adjust the pH to about 3 with ammonia TS added dropwise. Then, add 0.5 mL of Cu-PAN TS, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS while boiling until the color of the solution changes from red to yellow lasting for more than 1 minute. Perform a blank determination.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 0.2698 mg of Al

**Standard Ammonium Solution** Dissolve 2.97 g of ammonium chloride, exactly weighed, in water for ammonium limit test to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water for ammonium limit test to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of ammonium (NH<sub>4</sub><sup>+</sup>).

**Standard Arsenic Solution** See Arsenic Limit Test <1.11>.

**Standard Arsenic Stock Solution** See Arsenic Limit Test <1.11>.

**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 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.5 μg of boron (B).

**Standard Cadmium Solution** Measure exactly 10 mL of Standard Cadmium Stock Solution, and add diluted nitric acid (1 in 3) to make exactly 1000 mL. Pipet 10 mL of this solution, and add diluted nitric acid (1 in 3) to make 100 mL. Each mL 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 100 mL of dilute nitric acid by gentle heating, cool, and add dilute nitric acid to make exactly 1000 mL.

**Standard Calcium Solution** Weigh exactly 0.250 g of calcium carbonate, add 5 mL of dilute hydrochloric acid and 25 mL of water, and dissolve by heating. After cooling, add

water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of calcium (Ca).

**Standard Calcium Solution for Atomic Absorption Spectrophotometry** Weigh accurately 0.250 g of calcium carbonate, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of calcium (Ca).

**Standard Copper Solution** Pipet 10 mL of Standard Copper Stock Solution, and dilute with water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of copper (Cu). Prepare before use.

**Standard Copper Stock Solution** Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

**Standard Cyanide Solution** Measure exactly a volume of Standard Cyanide Stock Solution, equivalent to 10 mg of cyanide (CN), add 100 mL of sodium hydroxide TS and water to make exactly 1000 mL. Each mL 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 1000 mL. Measure exactly 100 mL of this solution, add 0.5 mL of 4-dimethylaminobenzylidene rhodanine TS, and titrate <2.50> 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 Oxygen Flask Combustion Method <1.06>.

**Standard Gold Solution for Atomic Absorption Spectrophotometry** To 25 mL of Standard Gold Stock Solution, exactly measured, add water to make exactly 1000 mL. Each mL of this solution contains 0.025 mg of gold (Au).

**Standard Gold Stock Solution** Dissolve 0.209 g of hydrogen tetrachloroaurate (III) tetrahydrate, exactly weighed, in 2 mL of aqua regia, heat on a water bath for 10 minutes, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of gold (Au).

**Standard Iron Solution** Weigh exactly 86.3 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in 100 mL of water, and add 5 mL of dilute hydrochloric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of iron (Fe).

**Standard Iron Solution for Atomic Absorption Spectrophotometry** To exactly 5 mL of Standard Iron Stock Solution add water to make exactly 200 mL. Prepare before use. Each mL of this solution contains 0.250 mg of iron (Fe).

**Standard Iron Stock Solution** Dissolve exactly 4.840 g of iron (III) chloride hexahydrate in diluted hydrochloric acid (9 in 25) to make exactly 100 mL.

**Standard Lead Solution** Measure exactly 10 mL of Standard Lead Stock Solution, and add water to make exactly 100 mL. Each mL 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 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.

**Standard Liquids for Calibrating Viscosimeters** [JIS, Standard Liquids for Calibrating Viscosimeters (Z 8809)]

**Standard Magnesium Solution for Atomic Absorption Spectrophotometry** To exactly 1 mL of Standard Magnesium Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.0100 mg of magnesium (Mg).

**Standard Magnesium Stock Solution** Dissolve exactly 8.365 g of magnesium chloride hexahydrate in 2 mol/L hydrochloric acid TS to make exactly 1000 mL.

**Standard Mercury Solution** Weigh exactly 13.5 mg of mercury (II) chloride, previously dried for 6 hours in a desiccator (silica gel), dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add 10 mL of dilute nitric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.1  $\mu$ g of mercury (Hg). Prepare before use.

**Standard Methanol Solution** See Methanol Test <1.12>.

**Standard Nickel Solution** Dissolve 6.73 g of ammonium nickel (II) sulfate hexahydrate, exactly weighed, in water to make exactly 1000 mL. Pipet 5 mL of this solution, add water to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of nickel (Ni).

**Standard Nitric Acid Solution** Weigh exactly 72.2 mg of potassium nitrate, dissolve in water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of nitrogen (N).

**Standard Phosphoric Acid Solution** Weigh exactly 0.358 g of potassium dihydrogen phosphate, previously dried to constant mass in a desiccator (silica gel), and add 10 mL of diluted sulfuric acid (3 in 10) and water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.025 mg of phosphoric acid (as PO<sub>4</sub>).

**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 1000 mL. Each mL of this solution contains 5.00 mg of potassium (K).

**Standard Selenium Solution** To exactly 1 mL of Standard Selenium Stock Solution add water to make exactly 1000 mL. Prepare before use. It contains 1.0  $\mu$ g of selenium (Se) per mL.

**Standard Selenium Stock Solution** Dissolve exactly 1.405 g of selenium dioxide in 0.1 mol/L nitric acid to make exactly 1000 mL.

**Standard Silver Solution for Atomic Absorption Spectrophotometry** Measure exactly 10 mL of Standard Silver Stock Solution, and add water to make exactly 1000 mL. Each mL 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 1000 mL. Each mL 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 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of sodium dodecylbenzene sulfonate [CH<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>Na].

**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 1000 mL. Each mL of this solution contains 1.00 mg of sodium (Na).

**Standard Tin Solution** Weigh exactly 0.250 g of tin, and dissolve in 10 mL of sulfuric acid by heating. After cooling, transfer this solution with 400 mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500 mL. Pipet 10 mL of this solution, and add diluted hydrochloric acid (1 in 5) to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of tin (Sn). Prepare before use.

**Standard Vinyl Chloride Solution** Transfer about 190 mL of ethanol for gas chromatography into a 200-mL 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 liquidized, 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 200 mL. Pipet 1 mL of this solution, add ethanol for gas chromatography, cooled previously in a methanol-dry ice bath to make exactly 100 mL. Preserve in a hermetic container, at a temperature not exceeding -20°C. This solution contains 10  $\mu$ g of vinyl chloride per mL.

**Standard Water-Methanol Solution** See Water Determination <2.48>.

**Standard Zinc Solution** Measure exactly 25 mL of Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.025 mg of zinc (Zn).

**Standard Zinc Solution for Atomic Absorption Spectrophotometry** See Test for Rubber Closure for Aqueous Infusions <7.03>.

**Standard Zinc Stock Solution** Dissolve exactly 1.000 g of zinc (standard reagent), in 100 mL of water and 5 mL of hydrochloric acid with the aid of gentle heating, cool, and add water to make exactly 1000 mL.

## 9.23 Matching Fluids for Color

Matching Fluids for Color are used as the reference for the comparison of color in a text of the Pharmacopoeia.

They 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 otherwise specified, transfer both

Table 9.23-1 Matching fluid for color

Matching fluid for color	Parts of cobalt (II) Chloride CS (mL)	Parts of iron (III) Chloride CS (mL)	Parts of copper (II) Sulfate CS (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

solutions and fluids to Nessler tubes and view transversely against a white background.

**Cobalt (II) Chloride CS** Weigh 65 g of cobalt (II) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of the solution, add 75 mL of water and 0.05 g of murexide-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 <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes, after the addition of 0.2 mL of diluted ammonia solution (28) (1 in 10) near the endpoint, from yellow to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 2.379 mg of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 59.5 mg of cobalt (II) chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ : 237.93) in each mL, and use this solution as the colorimetric stock solution.

**Cobaltous Chloride CS** See Cobalt (II) Chloride CS.

**Copper (II) Sulfate CS** Weigh 65 g of copper (II) sulfate pentahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 2 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator. Titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from green to pur-

ple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 2.497 mg of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of copper (II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ : 249.69) in each mL, and use this solution as the colorimetric stock solution.

**Copper Sulfate CS** See Copper (II) Sulfate CS.

**Iron (III) Chloride CS** Weigh 55 g of iron (III) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, transfer to an iodine flask, add 15 mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water to the mixture, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 27.03 mg of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ : 270.30) in each mL, and use this solution as the colorimetric stock solution.

**Matching Fluids for Color** Measure exactly the volume of colorimetric stock solutions and water shown in the following table with a buret or a pipet graduated to less than 0.1 mL, and mix.

## Reagents, Test Solutions, etc.

### 9.41 Reagents, Test Solutions

Reagents are the substances used in the tests of the Pharmacopoeia. The reagents that are described as "Standard reagent for volumetric analysis", "Special class", "First class", "For water determination", etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS). The tests for them are performed according to the test methods of JIS. In the case where the reagent name in the Pharmacopoeia differs from that of JIS, the JIS name is given in the brackets. The reagents for which a monograph's title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagents that are described merely as test items, the corresponding test method of the Pharmacopoeia is applied.

Test Solutions are the solutions prepared for use in the tests of the Pharmacopoeia.

**Acemetacin**  $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$  [Same as the namesake monograph]

**Acemetacin for assay**  $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$  [Same as the monograph Acemetacin. When dried, it contains not less than 99.5% of acemetacin ( $\text{C}_{21}\text{H}_{18}\text{ClNO}_6$ ) meeting the following additional specifications.]

**Purity** Related substances—Dissolve 40 mg of acemetacin for assay in 10 mL of methanol, and use this solution as

the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than acetaminophen obtained from the sample solution is not larger than 1/2 times the peak area of acetaminophen obtained from the standard solution, and the total area of the peaks other than the peak of acetaminophen is not larger than the peak area of acetaminophen from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Acetaminophen Tablets.

Time span of measurement: About 4 times as long as the retention time of Acetaminophen.

#### System Suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of acetaminophen obtained from 10  $\mu$ L of this solution is equivalent to 3 to 7% of that of acetaminophen from 10  $\mu$ L of the standard solution.

System performance: Dissolve 75 mg of acetaminophen and 75 mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of a solution of hexyl parahydroxybenzoate in methanol (1 in 250), and add methanol to make 50 mL. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, acetaminophen, indometacin and hexyl parahydroxybenzoate are eluted in this order with the resolutions between the peaks of acetaminophen and indometacin and between the peaks of indometacin and hexyl parahydroxybenzoate being not less than 3, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acetaminophen is not more than 1.5%.

**Acenaphthene**  $C_{12}H_{10}$  White to pale yellowish white crystals or crystalline powder, having a characteristic aroma. Freely soluble in diethyl ether and in chloroform, soluble in acetonitrile, sparingly soluble in methanol, and practically insoluble in water.

*Identification*—Determine the infrared absorption spectrum of acenaphthene according to the paste method under Infrared Spectrophotometry <2.25>, with 5 mg of acenaphthene: it exhibits absorption at the wave numbers of about 1605  $cm^{-1}$ , 840  $cm^{-1}$ , 785  $cm^{-1}$  and 750  $cm^{-1}$ .

*Melting point* <2.60>: 93 – 96°C

*Purity*—Dissolve 0.1 g of acenaphthene in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to 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: Hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 150- to 180- $\mu$ m siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M.

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  $\mu$ L of the solution prepared by adding chloroform to 1.0 mL of the sample 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 acenaphthene beginning after the solvent peak.

*Residue on ignition* <2.44>—Not more than 0.1% (1 g).

**Acetal**  $C_6H_{14}O_2$  A clear and colorless volatile liquid. Miscible with water and with ethanol (95).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.382

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.824

*Boiling point* <2.57>: about 103°C

**Acetaldehyde**  $CH_3CHO$  [K 8030, First class]

**Acetaldehyde for assay** Distil 100 mL of acetaldehyde under reduced pressure, discard the first 20 mL of the distillate, and use the subsequent. Prepare before use.

**Acetaldehyde for gas chromatography**  $C_2H_4O$  A clear and colorless, flammable liquid. Miscible with water and with ethanol (95).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.332

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.788

*Boiling point* <2.57>: about 21°C

**2-Acetamidoglutarimide**  $C_7H_{10}N_2O_3$ ; 170.17

*Identification*—Determine the infrared absorption spectrum of 2-acetamidoglutarimide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3350  $cm^{-1}$ , 1707  $cm^{-1}$ , 1639  $cm^{-1}$  and 1545  $cm^{-1}$ .

*Purity* Related substances—Dissolve 10 mg of 2-acetamidoglutarimide in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 20  $\mu$ L each of the sample solution and standard solution as directed in the Purity (3) under Acetylglutamide Aluminum: the total of the peak areas other than 2-acetamidoglutarimide from the sample solution is not larger than the peak area from the standard solution.

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 20 mg of 2-acetamidoglutarimide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS  
= 0.8509 mg of  $C_7H_{10}N_2O_3$

**Acetaminophen**  $C_8H_9NO_2$  [Same as the namesake monograph]

**Acetanilide**  $C_8H_9NO$  White, crystals or crystalline powder.

*Melting point* <2.60>: 114 – 117°C

***p*-Acetanisidide** C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> 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* <2.60>: 126 – 132°C

*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 Gas Chromatography <2.02> according to the following conditions, and determine 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 50 mL per minute 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 beginning after the solvent peak.

**Acetate buffer solution, pH 3.5** Dissolve 50 g of ammonium acetate in 100 mL of 6 mol/L hydrochloric acid TS, adjust to pH 3.5 with ammonia TS or 6 mol/L hydrochloric acid TS, if necessary, and add water to make 200 mL.

**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.

**0.01 mol/L Acetate buffer solution, pH 5.0** Dissolve 385 g of ammonium acetate in 900 mL of water, add acetic acid (31) to adjust the pH to 5.0, and then add water to make 1000 mL.

**Acetate buffer solution, pH 5.4** To 5.78 mL of acetic acid (100) add water to make 1000 mL (solution A). Dissolve 8.2 g of anhydrous sodium acetate in water to make 1000 mL (solution B). Mix 176 mL of the solution A and 824 mL of the solution B, and adjust, if necessary, the pH to 5.4 with the solution A or the solution B.

**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 1000 mL.

**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 buffer solution containing 0.1% bovine serum albumin** Dissolve 0.1 g of bovine serum albumin in sodium acetate trihydrate solution (1 in 100) to make exactly 100 mL, and adjust the pH to 4.0 with 1 mol/L hydrochloric acid TS.

**Acetic acid, dilute** Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

**Acetic acid for nonaqueous titration** [K 8355, Special class. 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 sample solution. Pipet 25 mL of the sample solution, titrate <2.50> 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 sample solution, add 75 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL) (potentiometric titration). A – B is not more than 0.1 mL (not more than 0.001 g/dL).

**Acetic acid, glacial** See acetic acid (100).

**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 4.0 with acetic acid (100), and add water to make 1000 mL.

**0.05 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0** To 3.0 g of acetic acid (100) add water to make 1000 mL. Adjust to pH 4.0 with a solution prepared by dissolving 3.4 g of sodium acetate trihydrate in water to make 500 mL.

**0.1 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0** Dissolve 13.61 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.0 with acetic acid (100), 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.

**0.05 mol/L Acetic acid-sodium acetate buffer solution, pH 4.6** Dissolve 6.6 g of sodium acetate trihydrate in 900 mL of water, and add 3 mL of acetic acid and water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 4.7** Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

**Acetic acid-sodium acetate buffer solution, pH 5.0** To 140 mL of sodium acetate TS add 60 mL of dilute acetic acid

and water to make 1000 mL.

**1 mol/L Acetic acid-sodium acetate buffer solution, pH 5.0** To sodium acetate TS add dilute acetic acid, and adjust the pH to 5.0.

**Acetic acid-sodium acetate buffer solution, pH 5.5** Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

**Acetic acid-sodium acetate buffer solution, pH 5.6** Dissolve 12 g of sodium acetate trihydrate in 0.66 mL of acetic acid (100) and water to make 100 mL.

**Acetic acid-sodium acetate TS** Mix 17 mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

**Acetic acid-sodium acetate TS, pH 7.0** Dissolve 4.53 g of sodium acetate trihydrate in water to make 100 mL, and adjust the pH to 7.0 with diluted acetic acid (100) (1 in 50).

**0.02 mol/L Acetic acid-sodium acetate TS** Dissolve 2.74 g of sodium acetate trihydrate in a suitable amount of water, and add 2 mL of acetic acid (100) and water to make 1000 mL.

**0.25 mol/L Acetic acid TS** To 3 g of acetic acid (100) add water to make 200 mL.

**6 mol/L Acetic acid TS** Dilute 36 g of acetic acid (100) with water to make 100 mL.

**Acetic acid (100)**  $\text{CH}_3\text{COOH}$  [K 8355, Acetic Acid, Special class]

**Acetic acid (100)-sulfuric acid TS** To 5 mL of acetic acid (100) add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

**Acetic acid (31)** Dilute 31.0 g of acetic acid (100) with water to make 100 mL (5 mol/L).

**Acetic anhydride**  $(\text{CH}_3\text{CO})_2\text{O}$  [K 8886, Special class]

**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**  $\text{CH}_3\text{COCH}_3$  [K 8034, Special class]

**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, distill, and dehydrate with freshly ignited anhydrous potassium carbonate. Distill by using a fractionating column under protection from moisture, and collect the fraction distilling at 56°C.

**Acetone for purity of crude drug**  $\text{CH}_3\text{COCH}_3$  [K 8034, Acetone, Special class] Use acetone meeting the following additional specification. Evaporate 300.0 mL of acetone to be tested in vacuum at a temperature not higher than 40°C, add the acetone to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Further pipet 2 mL of

this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of  $\gamma$ -BHC beginning from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained from 1  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC from 1  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of  $\gamma$ -BHC beginning after the solvent peak.

**Acetonitrile**  $\text{CH}_3\text{CN}$  [K 8032, Special class]

**Acetonitrile for liquid chromatography**  $\text{CH}_3\text{CN}$  Colorless and clear liquid. Mixable with water.

**Purity** Ultraviolet light absorbing substances—Determine the absorbances at the following wavelengths as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control: not more than 0.07 at 200 nm, not more than 0.046 at 210 nm, not more than 0.027 at 220 nm, not more than 0.014 at 230 nm and not more than 0.009 at 240 nm.

**Acettrizoic acid**  $\text{C}_9\text{H}_6\text{I}_3\text{NO}_3$  White powder.

**Purity** Related substances—Dissolve 60 mg of acettrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the sample solution. Proceed the test with 5  $\mu\text{L}$  of the sample solution as directed in the Assay under Meglumine Sodium Amidotrizoate Injection: any peaks other than the principal peak are not observed.

**Acetylacetone**  $\text{CH}_3\text{COCH}_2\text{COCH}_3$  [K 8027, Special class]

**Acetylacetone TS** Dissolve 150 g of ammonium acetate in a sufficient quantity of water, and add 3 mL of acetic acid (100), 2 mL of acetylacetone and water to make 1000 mL. Prepare before use.

**Acetylene** See dissolved acetylene.

**Achyranthes root for thin-layer chromatography** A heat-dried, pulverized root of *Achyranthes fauriei* Leveillé et Vaniot (*Amaranthaceae*) meeting the following additional specifications.

**Identification** (1) To 2 g of pulverized achyranthes root for thin-layer chromatography add 10 mL of water, shake for 10 minutes, add 5 mL of 1-butanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chikuset-

susaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the standard solution shows a deep purplish red spot at around Rf value of 0.35, and the sample solution shows spots equivalent to those described below:

Rf value	Color and shape of the spot
Around 0	A weak spot, black
Around 0.1	A weak spot, strong purplish red
Around 0.2	A weak, tailing spot, strong purplish red
Around 0.25	A strong spot, deep purplish red
Around 0.35	A leading spot, deep purplish red
Around 0.45	A weak spot, dull yellow
Around 0.5	A weak spot, grayish purplish red
Around 0.75	A weak spot, grayish red
Around 0.9	A weak spot, dull red

(2) Perform the test as directed in the operating conditions under (1), except using a mixture of 1-propanol, ethyl acetate and water (4:4:3) as the developing solvent: the standard solution shows a deep purplish red spot at around Rf value of 0.45, and the sample solution shows spots equivalent to those described below:

Rf value	Color and shape of the spot
Around 0.25	A weak spot, strongly purplish red
Around 0.25 – 0.3	A leading spot or two strong spots, strongly purplish red
Around 0.35	A deep purplish red spot
Around 0.4	A weak spot, dull red
Around 0.42	A dark red spot
Around 0.45	A weak spot, grayish red
Around 0.65	A weak spot, dull greenish yellow
Around 0.7	A weak spot, grayish red
Around 0.85	A weak spot, grayish red
Around 0.95	A weak spot, dull yellow-red

**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.

**Aconitine for purity**  $\text{C}_{34}\text{H}_{47}\text{NO}_{11}$  White, crystals or crystalline powder. Sparingly soluble in acetonitrile and in ethanol (99.5), slightly soluble in diethyl ether, and practi-

cally insoluble in water. Melting point: about 185°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of aconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500  $\text{cm}^{-1}$ , 1718  $\text{cm}^{-1}$ , 1278  $\text{cm}^{-1}$ , 1111  $\text{cm}^{-1}$ , 1097  $\text{cm}^{-1}$  and 717  $\text{cm}^{-1}$ .

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 211 – 243 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

**Purity** Related substances—

(1) Dissolve 5.0 mg of aconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of aconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of aconitine and the solvent obtained with the sample solution is not larger than the peak area of aconitine with the standard solution.

**Operating conditions**

**Detector, column, and column temperature:** Proceed as directed in the operating conditions in the Purity under Processed Aconitine Root.

**Mobile phase:** A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

**Flow rate:** Adjust the flow rate so that the retention time of aconitine is about 26 minutes.

**Time span of measurement:** About 3 times as long as the retention time of aconitine.

**System suitability**

**Test for required detectability:** Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of aconitine obtained from 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that obtained from 10  $\mu\text{L}$  of the standard solution.

**System performance:** Dissolve 1 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu\text{L}$  of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aconitine is not more than 1.5%.

*Water* <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

**Aconitum diester alkaloids standard TS for purity** It is a solution containing 10 mg of aconitine for purity, 10 mg of jesaconitine for purity, 30 mg of hypaconitine for purity and 20 mg of mesaconitine for purity in 1000 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). When proceed the test with 20  $\mu$ L of this solution as directed in the Purity under Processed Aconite Root at the detection wavelength 231 nm, the peaks of aconitine, jesaconitine, hypaconitine and mesaconitine are observed, and the ratio of their peak heights is about 10:1:35:30. When proceed the test at the detection wavelength 254 nm, the peaks of aconitine, jesaconitine, hypaconitine and mesaconitine are observed, and the ratio of their peak heights is about 2:8:7:6.

**Aconitum monoester alkaloids standard TS for assay** Weigh accurately about 20 mg of benzoylmesaconine hydrochloride for assay (separately, determine the water), about 10 mg of benzoylhypaconine hydrochloride for assay (separately, determine the water) and about 20 mg of 14-anisoylaconine hydrochloride for assay (separately, determine the water), dissolve in a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17) to make exactly 1000 mL. Perform the test with 20  $\mu$ L of this solution as directed in the Purity under benzoylmesaconine hydrochloride for assay: the peaks of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine appear with a peak area ratio of about 2:1:2.

**Aconitum monoester alkaloids standard TS for component determination** See aconitum monoester alkaloids standard TS for assay.

**Acrinol** See acrinol hydrate.

**Acrinol hydrate**  $C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$  [Same as the namesake monograph]

**Acrylamide**  $CH_2CHCONH_2$  White or pale yellow crystalline powder.

*Melting point* <2.60>: 83 – 87°C

*Content*: not less than 97.0%.

**Activated alumina** Aluminum oxide with specially strong adsorptive activity.

**Activated charcoal** [Same as the monograph Medicinal Carbon]

**Activated thromboplastin-time assay reagent** It is prepared by lyophilization of phospholipid (0.4 mg/mL) which is suspended in 1 mL of a solution of *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (61 in 5000), mixed with both silica-gel (4.3 mg/mL) and dextran after the extraction and purification from rabbit brain. Activated thromboplastin-time: 25 – 45 seconds (as assayed with human normal plasma).

**Activated thromboplastin-time assay TS** Dissolve an aliquot of activated thromboplastin-time assay reagent equivalent to 0.4 mg of phospholipid in 1 mL of water.

**Adipic acid**  $C_4H_8(COOH)_2$  White crystals or crystalline

powder. Freely soluble in ethanol (95), and sparingly soluble in water.

*Melting point* <2.60>: 151 – 154°C

*Content*: not less than 98.0%. Assay—Weigh accurately about 1 g of adipic acid, and 100 mL of water, dissolve by warming, cool, and titrate <2.50> 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** [K 8263, Special class. Same as the monograph 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 10 mL 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, reprepare by dissolving with the aid of heat.

**Ajmaline for assay**  $C_{20}H_{26}N_2O_2$  [Same as the monograph Ajmaline. When dried, it contains not less than 99.0% of ajmaline ( $C_{20}H_{26}N_2O_2$ ).]

**Alacepril**  $C_{20}H_{26}N_2O_5S$  [Same as the namesake monograph]

**Alacepril for assay** [Same as the monograph Alacepril. When dried, it contains not less than 99.0% of alacepril ( $C_{20}H_{26}N_2O_5S$ ).]

**$\beta$ -Alanine**  $C_3H_7NO_2$  Colorless crystals or a white crystalline powder. Freely soluble in water, very slightly soluble in methanol, and practically insoluble in ethanol (99.5) and in diethyl ether.

*Purity* Related substances—Dissolve 5.0 mg in 10 mL of diluted methanol (4 in 5), and use this as the sample solution. Pipet 1 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (5:2:2) to a distance of about 8 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**L-Alanine**  $C_3H_7NO_2$  [K 9101, Special class]

**Albiflorin**  $C_{23}H_{28}O_{11} \cdot xH_2O$  White powder having no odor. Freely soluble in water, in methanol and in ethanol (99.5).

*Identification*—Determine the absorption spectrum of a solution of albiflorin in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 230 nm and 234 nm.

*Purity* (1) Related substances 1—Dissolve 1 mg of albiflorin in 1 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot which appears at around *Rf* 0.2 does not appear.

(2) Related substances 2—Dissolve 1 mg of albiflorin in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Peony Root, and measure the peak areas about 2 times as long as the retention time of peoniflorin: the total area of the peaks other than albiflorin from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

**Albumin TS** Carefully separate the white from the yolk of a fresh hen's egg. Shake the white with 100 mL of water until the mixture is thoroughly mixed, and filter. Prepare before use.

**Alcian blue 8 GX**  $C_{56}H_{68}Cl_{14}CuN_{16}S_4$  Dark blue-purple powder.

**Alcian blue staining solution** Dissolve 0.5 g of alcian blue 8 GX in 100 mL of diluted acetic acid (100) (3 in 100).

**Aldehyde dehydrogenase** Each mg contains not less than 2 enzyme activity units. White powder.

**Assay**—Dissolve about 20 mg 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 sample solution. In a spectrophotometric cell, place 2.50 mL of pyrophosphate buffer solution, pH 9.0, 0.20 mL of a solution prepared by dissolving 20.0 mg of  $\beta$ -nicotinamide adenine dinucleotide (NAD) to make exactly 1 mL, 0.10 mL of a pyrazole solution (17 in 2500) and 0.10 mL of the sample solution, stir, stopper tightly, and allow to stand at  $25 \pm 1^\circ\text{C}$  for 2 minutes. To this solution add 0.01 mL of an acetaldehyde solution (3 in 1000), stir, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate a change ( $\Delta A$ ) 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  $\mu$ mol of acetaldehyde per minute when the test is conducted under the conditions of the Procedure.

Enzyme activity unit (unit/mg) of aldehyde dehydrogenase

$$= \frac{2.91 \times \Delta A \times 200}{6.3 \times M \times 0.10 \times 1000}$$

*M*: Amount (g) of sample

**Aldehyde dehydrogenase TS** Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10 mL of water. Prepare before use.

**Aldehyde-free ethanol** See ethanol, aldehyde-free.

**Alendronate sodium hydrate**  $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$  [Same as the namesake monograph]

**Alisol A for thin-layer chromatography**  $C_{30}H_{50}O_5$  A white to pale yellow powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $+86 - +106^\circ$  (5 mg previously dried on silica gel for 24 hours, methanol, 1 mL, 50 mm).

**Purity** Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 5  $\mu$ L of this solution as directed in the Identification (6) under Saireito Extract: no

spot appears other than the principal spot of around *Rf* 0.3.

**Alizarin complexone**  $C_{19}H_{15}NO_8$  (1,2-Dihydroxyanthra-quinone-3-ylmethylamine-*N,N*-diacetate) A yellow-brown powder. Soluble in ammonia TS, and practically insoluble in water, in ethanol (95) and in diethyl 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 10 mL of this solution add acetic acid-potassium acetate buffer solution, pH 4.3, to make 100 mL. Place 1 drop of this solution on a white spot plate, add 1 drop of a solution of sodium fluoride (1 in 100,000) and 1 drop of cerium (III) nitrate 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 (1 in 50), then add 800 mL of water and 0.2 g of sodium acetate trihydrate, and dissolve. Adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid VS, and add water to make 1000 mL.

**Alizarin red S**  $C_{14}H_7NaO_7S$  [K 8057, Special class]

**Alizarin red S TS** Dissolve 0.1 g of alizarin red S in water to make 100 mL, and filter if necessary.

**Alizarin S** See alizarin red S.

**Alizarin S TS** See alizarin red S TS.

**Alizarin yellow GG**  $C_{13}H_8N_3NaO_5$  [K 8056, Special class]

**Alizarin yellow GG-thymolphthalein TS** Mix 10 mL of alizarin GG TS with 20 mL of thymolphthalein TS.

**Alizarin yellow GG TS** Dissolve 0.1 g of alizarin yellow GG in 100 mL of ethanol (95), and filter if necessary.

**Alkali copper TS** Dissolve 70.6 g of disodium hydrogen phosphate dodecahydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600 mL of water, and add 20 mL of a solution of sodium hydroxide (1 in 5). To this mixture add, with stirring, 100 mL of a solution of copper (II) sulfate (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate VS and water to make 1000 mL.

**Alkaline blue tetrazolium TS** See blue tetrazolium TS, alkaline.

**Alkaline copper solution** See alkaline copper TS for protein content determination.

**Alkaline copper TS** Dissolve 2 g of anhydrous sodium carbonate in 100 mL of 0.1 mol/L sodium hydroxide TS. To 50 mL of this solution add 1 mL of a mixture of a solution of copper (II) sulfate pentahydrate (1 in 100) and a solution of potassium tartrate (1 in 50) (1:1), and mix.

**Alkaline copper TS for protein content determination** Dissolve 0.8 g of sodium hydroxide in water to make 100 mL. Dissolve 4 g of anhydrous sodium carbonate in this solution to make solution A. Combine 1 mL of copper (II) sulfate pentahydrate solution (1 in 50) and 1 mL of sodium tar-

trate dihydrate solution (1 in 25) to make solution B. Mix 50 mL of solution A and 1 mL of solution B. Prepare at the time of use.

**Alkaline copper (II) sulfate solution** See copper (II) sulfate solution, alkaline.

**Alkaline copper (II) TS** Dissolve 20 g of anhydrous sodium carbonate in dilute sodium hydroxide TS to make 1000 mL, and use this solution as solution A. Dissolve 0.5 g of copper (II) sulfate pentahydrate in potassium sodium tartrate tetrahydrate solution (1 in 100) to make 100 mL, and use this solution as solution B. To 50 mL of solution A add 1 mL of solution B. Prepare before use.

**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 hydroxylamine TS** See hydroxylamine TS, alkaline.

**Alkaline *m*-dinitrobenzene TS** See 1,3-dinitrobenzene TS, alkaline.

**Alkaline phosphatase** Obtained from bovine small intestine, a white to grayish white or yellow-brown, freeze-dried powder having no odor.

Alkaline phosphatase contains not less than 1 unit per mg and no salts. One unit of alkaline phosphatase indicates an amount of the enzyme which produces 1  $\mu$ mol of 4-nitrophenol in 1 minute at 37°C and pH 9.8, from 4-nitrophenylphosphate ester used as the substrate.

**Alkaline phosphatase TS** Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

**Alkaline picric acid TS** See 2,4,6-trinitrophenol TS, alkaline.

**Alkaline potassium ferricyanide TS** See potassium hexacyanoferrate (III) TS, alkaline.

**Alkylene glycol phthalate ester for gas chromatography** Prepared for gas chromatography.

**Allopurinol**  $C_5H_4N_4O$  [Same as the namesake monograph]

**Allopurinol for assay**  $C_5H_4N_4O$  [Same as the monograph Allopurinol. When dried, it contains not less than 99.0% of allopurinol ( $C_5H_4N_4O$ ).]

**Alminoprofen for assay**  $C_{13}H_{17}NO_2$  [Same as the monograph Alminoprofen. When dried, it contains not less than 99.5% of alminoprofen ( $C_{13}H_{17}NO_2$ ).]

**Alternative thioglycolate medium** See Sterility Test <4.06> under the General Tests, Processes and Apparatus.

**Aluminon**  $C_{22}H_{23}N_3O_9$  [K 8011, 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 [K 8069, Special class]

**Aluminum chloride** See aluminum (III) chloride hexahydrate.

**Aluminum chloride TS** See Aluminum (III) chloride TS.

**Aluminum (III) chloride TS** Dissolve 64.7 g of aluminum (III) chloride hexahydrate in 71 mL of water, add 0.5 g of activated charcoal, then shake for 10 minutes, and filter. Adjust the pH of the filtrate to 1.5 with a solution of sodium hydroxide (1 in 100) with stirring, and filter if necessary.

**Aluminum (III) chloride hexahydrate**  $AlCl_3 \cdot 6H_2O$   
[K 8114, Special class]

**Aluminum oxide**  $Al_2O_3$  White crystals, crystalline powder, or powder. Boiling point: about 3000°C. Melting point: about 2000°C.

**Aluminum potassium sulfate dodecahydrate**  
 $AlK(SO_4)_2 \cdot 12H_2O$  [K 8255, Special class]

**6-Amidino-2-naphthol methanesulfonate**  
 $C_{11}H_{10}N_2O \cdot CH_4O_3S$  A white to pale yellow crystalline powder. Melting point: about 233°C (with decomposition).

**Purity**—A solution obtained by dissolving 0.5 g of 6-amidino-2-naphthol methanesulfonate in 10 mL of methanol is clear.

**Amidosulfuric acid (standard reagent)**  $HOSO_2NH_2$   
[K 8005, Standard substance for volumetric analysis]

**Amidotrizoic acid for assay**  $C_{11}H_9I_3N_2O_4$  [Same as the monograph Amidotrizoic Acid] It contains not less than 99.0% of amidotrizoic acid ( $C_{11}H_9I_3N_2O_4$ ), calculated on the dried basis.

***p*-Aminoacetophenone** See 4-aminoacetophenone.

***p*-Aminoacetophenone TS** See 4-aminoacetophenone TS.

**4-Aminoacetophenone**  $H_2NC_6H_4COCH_3$  Light yellow, crystals or crystalline powder, having a characteristic odor.  
**Melting point** <2.60>: 105 – 108°C

**4-Aminoacetophenone TS** Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

**4-Aminoantipyrine**  $C_{11}H_{13}N_3O$  [K 8048, Special class]

**4-Aminoantipyrine hydrochloride**  $C_{11}H_{13}N_3O \cdot HCl$   
Light yellow crystalline powder. It dissolves in water. Melting point: 232 – 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:** 100.6 – 108.5%. Assay—Weigh accurately about 0.5 g of 4-aminoantipyrine 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 <2.50> with 0.1 mol/L silver nitrate VS.

Each mL of 0.1 mol/L silver nitrate VS  
= 23.97 mg of  $C_{11}H_{13}N_3O \cdot HCl$

**4-Aminoantipyrine hydrochloride TS** Dissolve 1 g of 4-aminoantipyrine hydrochloride in water to make 50 mL.

**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.

**2-Aminobenzimidazole**  $C_7H_7N_3$  White to light yellow

crystals or crystalline powder. Melting point: about 231°C (with decomposition).

**Aminobenzoate derivatization TS** To 0.28 g of ethyl aminobenzoate add 600  $\mu\text{L}$  of methanol, warm at about 50°C to dissolve, and add 170  $\mu\text{L}$  of acetic acid and 145  $\mu\text{L}$  of borane-pyridine complex.

***p*-Aminobenzoic acid** See 4-aminobenzoic acid.

**4-Aminobenzoic acid**  $\text{H}_2\text{NC}_6\text{H}_4\text{COOH}$  White to very pale yellow crystalline powder.

**Purity** Clarity of solution—Dissolve 0.1 g of 4-aminobenzoic acid in 10 mL of ethanol (95): the solution is clear.

**2-Amino-1-butanol**  $\text{CH}_3\text{CH}_2\text{CH}(\text{NH}_2)\text{CH}_2\text{OH}$  Clear, colorless to light yellow liquid. Miscible with water and dissolves in methanol.

**Refractive index**  $\langle 2.45 \rangle n_D^{20}$ : 1.450 – 1.455

**Specific gravity**  $\langle 2.56 \rangle d_{20}^{20}$ : 0.944 – 0.950

**Purity** Related substances—Dissolve 50 mg of 2-amino-1-butanol in 10 mL of methanol, measured exactly, and perform the test with 2  $\mu\text{L}$  of this solution as directed in the Purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

**4-Aminobutylic acid**  $\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{COOH}$  White crystals or crystalline powder. Melting point: about 200°C (with decomposition).

**2-Amino-5-chlorobenzophenone for thin-layer chromatography**  $\text{C}_{13}\text{H}_{10}\text{ClNO}$  Yellow, crystalline powder.

**Melting point**  $\langle 2.60 \rangle$ : 97 – 101°C

**Purity** Related substances—Dissolve 10 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and perform the test with this solution as directed in the purity (2) under Chlordiazepoxide: any spot other than the principal spot at the *Rf* value about 0.7 does not appear.

**4-Amino-*N,N*-diethylaniline sulfate monohydrate**  $\text{H}_2\text{NC}_6\text{H}_4\text{N}(\text{C}_2\text{H}_5)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$  White to slightly colored powder. It dissolves in water.

**Melting point**  $\langle 2.60 \rangle$ : 173 – 176°C

**Residue on ignition**  $\langle 2.44 \rangle$ : not more than 0.1% (1 g).

**4-Amino-*N,N*-diethylaniline sulfate TS** Dissolve 0.2 g of 4-amino-*N,N*-diethylaniline sulfate monohydrate in water to make 100 mL. Prepare before use, protected from light.

**2-Aminoethanethiol hydrochloride**  $\text{H}_2\text{NCH}_2\text{CH}_2\text{SH} \cdot \text{HCl}$  White crystal or granule.

**Melting point**  $\langle 2.60 \rangle$ : 65 – 71°C

**2-Aminoethanol**  $\text{NH}_2\text{CH}_2\text{CH}_2\text{OH}$  [K 8109, Special class]

***N*-Aminohexamethyleneimine**  $(\text{CH}_2)_6\text{NNH}_2$  Clear, colorless to pale yellow liquid.

**Refraction index**  $\langle 2.45 \rangle n_D^{20}$ : 1.482 – 1.487

**Specific gravity**  $\langle 2.56 \rangle d_{20}^{20}$ : 0.936 – 0.942

**2-Amino-2-hydroxymethyl-1,3-propanediol**  $\text{C}_4\text{H}_{11}\text{NO}_3$  [K 9704, Special class]

**2-Amino-2-hydroxymethyl-1,3-propanediol hydrochloride**  $\text{C}_4\text{H}_{11}\text{NO}_3 \cdot \text{HCl}$  White crystals or crystalline powder

**4-(Aminomethyl)benzoic acid**  $\text{C}_8\text{H}_9\text{NO}_2$  A white pow-

der.

**Purity**—Dissolve 10 mg of 4-(aminomethyl)benzoic acid in 100 mL of water, and use this as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography  $\langle 2.01 \rangle$  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.

**1-Amino-2-methylnaphthalene**  $\text{C}_{11}\text{H}_{11}\text{N}$  Pale yellow to pale brown masses or liquid.

**2-Aminomethylpiperidine**  $\text{C}_6\text{H}_{14}\text{N}$  A colorless or light yellowish clear liquid, having an amine like characteristic odor.

**Identification**—Determine the infrared absorption spectrum as directed in the liquid film method under Infrared Spectrophotometry  $\langle 2.25 \rangle$ : it exhibits absorption at the wave numbers of about 3280  $\text{cm}^{-1}$ , 1600  $\text{cm}^{-1}$ , 1440  $\text{cm}^{-1}$ , 1120  $\text{cm}^{-1}$  and 840  $\text{cm}^{-1}$ .

**Purity** Related substances—Perform the test with 0.8  $\mu\text{L}$  of 2-aminomethylpiperidine as directed under Gas Chromatography  $\langle 2.02 \rangle$ . Determine each peak area by the automatic integration method, and calculate these amounts by the area percentage method: the total amount of the peaks other than 2-aminomethylpiperidine is not more than 1.5%.

**Operating conditions**

**Detector:** A hydrogen flame-ionization detector.

**Column:** A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 – 180  $\mu\text{m}$ ) coated with 10% of polyethylene glycol 20M for gas chromatography and 2% of potassium hydroxide.

**Column temperature:** 100°C at beginning, and increase to 200°C by 10°C per minute after injection.

**Carrier gas:** Nitrogen.

**Flow rate:** Adjust the flow rate so that the retention time of 2-aminomethylpiperidine is about 5 minutes.

**Time span of measurement:** About 2 times as long as the retention time of 2-aminomethylpiperidine.

**3-(2-Aminoethyl)indole**  $\text{C}_{10}\text{H}_{12}\text{N}_2$  Yellowish-brown crystals.

**Melting point**  $\langle 2.60 \rangle$ : about 118°C.

**1-Amino-2-naphthol-4-sulfonic acid**  $\text{C}_{10}\text{H}_9\text{NO}_4\text{S}$  [K 8050, 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.

***m*-Aminophenol** See 3-aminophenol.

**3-Aminophenol**  $\text{H}_2\text{NC}_6\text{H}_4\text{OH}$  White, crystals or crystalline powder.

**Melting point**  $\langle 2.60 \rangle$ : 121 – 125°C

**Content:** not less than 97.0%. **Assay**—Weigh accurately about 0.2 g, dissolve in 50 mL of acetic acid for nonaqueous titration, and titrate  $\langle 2.50 \rangle$  with 0.1 mol/L perchloric acid

VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 10.91 mg of  $\text{H}_2\text{NC}_6\text{H}_4\text{OH}$

***p*-Aminophenol hydrochloride** See 4-aminophenol hydrochloride.

**4-Aminophenol hydrochloride**  $\text{HOC}_6\text{H}_4\text{NH}_2\cdot\text{HCl}$   
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 50 mL of acetic acid for nonaqueous titration and 5 mL of mercury (II) acetate TS for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid-1,4-dioxane VS (indicator: 1 mL of *p*-naphtholbenzene TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS  
= 14.56 mg of  $\text{C}_6\text{H}_8\text{NOCl}$

*Storage*—Preserve in tight, light-resistant containers.

**Aminopropylsilanized silica gel for pretreatment** Prepared for pretreatment.

**Aminopyrine**  $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}$  White to pale yellow crystals or crystalline powder.

*Melting point* <2.60>: 107 – 109°C

**6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate**  
 $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_4$  Prepared for amino acid analysis or biochemistry.

**L-2-Aminosuberlic acid**  $\text{C}_8\text{H}_{15}\text{NO}_4$  White, crystals or crystalline powder. Odorless.

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : +19.1 – +20.1° (after drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm).

*Loss on drying* <2.41>: not more than 0.3% (1 g, 105°C, 2 hours).

*Assay*—Weigh accurately about 0.3 g of L-2-aminosuberlic acid, previously dried, add exactly 6 mL of formic acid to dissolve, then add exactly 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 18.92 mg of  $\text{C}_8\text{H}_{15}\text{NO}_4$

**Amiodarone hydrochloride for assay**  $\text{C}_{25}\text{H}_{29}\text{I}_2\text{NO}_3\cdot\text{HCl}$   
[Same as the monograph Amiodarone Hydrochloride. When dried, it contains not less than 99.5% of amiodarone hydrochloride ( $\text{C}_{25}\text{H}_{29}\text{I}_2\text{NO}_3\cdot\text{HCl}$ ).]

**Ammonia-ammonium acetate buffer solution, pH 8.0**  
To ammonium acetate TS add ammonia TS dropwise to 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 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-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) dropwise to adjust the pH to 10.0.

**Ammonia-ammonium chloride buffer solution, pH 10.7**  
Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonia 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 copper TS** To 0.5 g of cupric carbonate monohydrate add 10 mL of water, triturate, and add 10 mL of ammonia solution (28).

**Ammonia-ethanol TS** To 20 mL of ammonia solution (28) add 100 mL of ethanol (99.5).

**Ammonia gas**  $\text{NH}_3$  Prepare by heating ammonia solution (28).

**Ammonia-saturated 1-butanol TS** To 100 mL of 1-butanol add 60 mL of diluted ammonia solution (28) (1 in 100), shake vigorously for 10 minutes, and allow to stand. Use the upper layer.

**Ammonia solution (28)**  $\text{NH}_4\text{OH}$  [K 8085, Ammonia Water, Special class, Density: 0.90 g/mL, Content: 28 – 30%]

**Ammonia TS** To 400 mL of ammonia solution (28) add water to make 1000 mL (10%).

**1 mol/L Ammonia TS** To 65 mL of ammonia solution (28) add water to make 1000 mL.

**13.5 mol/L Ammonia TS** To exactly 9 mL of water add ammonia solution (28) to make exactly 50 mL.

**Ammonia water** See ammonia TS.

**1 mol/L Ammonia water** See 1 mol/L ammonia TS.

**13.5 mol/L Ammonia water** See 13.5 mol/L ammonia TS.

**Ammonia water, strong** See ammonia solution (28).

**Ammonium acetate**  $\text{CH}_3\text{COONH}_4$  [K 8359, Special class]

**Ammonium acetate TS** Dissolve 10 g of ammonium acetate in water to make 100 mL.

**0.5 mol/L Ammonium acetate TS** Dissolve 38.5 g of ammonium acetate in water to make 1000 mL.

**Ammonium amidosulfate**  $\text{NH}_4\text{OSO}_2\text{NH}_2$   
[K 8588, Special class]

**Ammonium amidosulfate TS** Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

**Ammonium aminetricloroplatinate for liquid chromatography**  $\text{Cl}_3\text{H}_7\text{N}_2\text{Pt}$  To 20 g of cisplatin add 600 mL of 6 mol/L hydrochloric acid TS, and heat under a reflux con-

denser for 4–6 hours to boil while stirring. After cooling, evaporate the solvent, and dry the orange residue at room temperature under reduced pressure. To the residue so obtained add 300 mL of methanol, and heat at about 50°C to dissolve. Filter, separate insoluble yellow solids, and wash the solids with 10 mL of methanol. Combine the filtrate and the washing, heat at about 50°C, and add slowly 100 mL of ethyl acetate while stirring. Cool the mixture to room temperature avoiding exposure to light, and allow to stand at –10°C for 1 hour. Filter the mixture to take off the formed crystals, wash the crystals with 100 mL of acetone, combine the washing to the filtrate, and evaporate to dryness to obtain orange crystals. If necessary, repeat the purification procedure described above to take off the insoluble crystals. To the orange crystals obtained add 300 to 500 mL of a mixture of acetone and methanol (5:1), and heat at about 50°C while stirring to dissolve. Filter while hot to take off the insoluble crystals, wash the crystals with the mixture, and combine the filtrate and washing. Repeat the procedure several times, and evaporate to dryness. Suspend the crystals so obtained in 50 mL of acetone, filter, wash the crystals with 20 mL of acetone, and dry the crystals at room temperature under reduced pressure. It is a yellow-brown crystalline powder.

**Identification**—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 80°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3480 cm<sup>-1</sup>, 3220 cm<sup>-1</sup>, 1622 cm<sup>-1</sup>, 1408 cm<sup>-1</sup> and 1321 cm<sup>-1</sup>.

**Purity** Related substances—Cisplatin Conduct this procedure using light-resistant vessels. Dissolve 10 mg in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of Cisplatin in *N,N*-dimethylformamide to make exactly 50 mL. Pipet 5 mL of this solution, add *N,N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of cisplatin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

**Operating conditions**

Proceed as directed in the operating conditions in the Assay under Cisplatin.

**System suitability**

**System performance:** When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 2500 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 5.0%.

**Ammonium aurintricarboxylate** See aluminon.

**Ammonium carbonate** [K 8613, 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<sub>4</sub>Cl [K 8116, 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 dihydrogenphosphate** NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>  
[K 9006, Special class]

**0.02 mol/L Ammonium dihydrogenphosphate TS**  
Dissolve 2.30 g of ammonium dihydrogen phosphate in water to make 1000 mL.

**Ammonium formate** HCOONH<sub>4</sub> Colorless crystals. Very soluble in water.

*Melting point* <2.60>: 116–119°C

**0.05 mol/L Ammonium formate buffer solution, pH 4.0**  
Dissolve 3.15 g of ammonium formate in 750 mL of water, adjust to pH 4.0 with formic acid, and add water to make 1000 mL.

**Ammonium hydrogen carbonate** NH<sub>4</sub>HCO<sub>3</sub> White or semi-transparency crystals, crystalline powder or masses, having an ammonia odor.

**Ammonium iron (II) sulfate hexahydrate**  
FeSO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>·SO<sub>4</sub>·6H<sub>2</sub>O [K 8979, Special class]

**Ammonium iron (III) citrate** [Same as the monograph Ferric Ammonium Citrate in the Japanese Standards of Food Additives]

**Ammonium iron (III) sulfate dodecahydrate**  
FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O [K 8982, Special class]

**Ammonium iron (III) sulfate TS** Dissolve 8 g of ammonium iron (III) sulfate dodecahydrate in water to make 100 mL.

**Ammonium iron (III) sulfate TS, acidic** Dissolve 20 g of ammonium iron (III) sulfate dodecahydrate 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 1 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

**Ammonium molybdate** See hexaammonium heptamolybdate tetrahydrate.

**Ammonium molybdate-sulfuric acid TS** See hexaammonium heptamolybdate-sulfuric acid TS

**Ammonium molybdate TS** See hexaammonium heptamolybdate TS.

**Ammonium nickel (II) sulfate** See ammonium nickel (II) sulfate hexahydrate.

**Ammonium nickel (II) sulfate hexahydrate**

$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  Green crystals or crystalline powder.

**Identification**—(1) Dissolve 1 g of ammonium nickel (II) sulfate hexahydrate in 20 mL of water, and use this as the sample solution. To 5 mL of the sample solution add 1 mL of barium chloride TS: a white precipitate is produced.

(2) To 5 mL of the sample solution obtained in (1) add 5 mL of 8 mol/L sodium hydroxide TS: a green precipitate is formed, and the liquid evolves ammonia on heating.

(3) To 5 mL of the sample solution obtained in (1) add 1 mL each of ammonia TS and dimethylglyoxime TS: a red precipitate is formed.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 1 g of ammonium nickel (II) sulfate hexahydrate, add 100 mL of water and 5 mL of ammonium chloride TS, then add exactly 20 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, warm to 50–60°C, add 10 mL of diluted ammonia solution (28) (1 in 2), and titrate with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution is changed from green to blue-purple (indicator: 0.05 g of murexide-sodium chloride indicator).

Each mL of disodium dihydrogen ethylenediamine tetraacetate VS

= 39.50 mg of  $(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$

**Ammonium nitrate**  $\text{NH}_4\text{NO}_3$  [K 8545, Special class]

**Ammonium oxalate** See ammonium oxalate monohydrate.

**Ammonium oxalate monohydrate**  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  [K 8521, Special class]

**Ammonium oxalate TS** Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/L).

**Ammonium peroxodisulfate**  $(\text{NH}_4)_2\text{S}_2\text{O}_8$  [K 8252, Special class]

**10% Ammonium peroxodisulfate TS** Dissolve 1 g of ammonium peroxodisulfate in water to make 10 mL.

**Ammonium persulfate** See ammonium peroxodisulfate.

**Ammonium polysulfide TS**  $(\text{NH}_4)_2\text{S}_x$  [K 8943, Ammonium Sulfide Solution (yellow), First class]

**Ammonium sodium hydrogenphosphate tetrahydrate**  $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$  [K 9013, Special class]

**Ammonium sulfamate** See ammonium amidosulfate.

**Ammonium sulfamate TS** See ammonium amidosulfate TS.

**Ammonium sulfate**  $(\text{NH}_4)_2\text{SO}_4$  [K 8960, Special class]

**Ammonium sulfate buffer solution** Dissolve 264 g of ammonium sulfate in 1000 mL of water, add 1000 mL of 0.5 mol/L sulfuric acid TS, shake, and filter. The pH of this solution is about 1.

**Ammonium sulfide TS**  $(\text{NH}_4)_2\text{S}$  [K 8943, Ammonium Sulfide Solution, (colorless), First class] Store in small, well-filled containers, protected from light.

**Ammonium tartrate** See L-ammonium tartrate.

**L-Ammonium tartrate**  $\text{C}_4\text{H}_{12}\text{N}_2\text{O}_6$  [K 8534, (+) Am-

monium tartrate, Special class]

**Ammonium thiocyanate**  $\text{NH}_4\text{SCN}$  [K 9000, Special class]

**Ammonium thiocyanate-cobalt (II) nitrate TS** Dissolve 17.4 g of ammonium thiocyanate and 2.8 g of cobalt (II) nitrate hexahydrate in water to make 100 mL.

**Ammonium thiocyanate TS** Dissolve 8 g of ammonium thiocyanate in water to make 100 mL (1 mol/L).

**Ammonium vanadate** See ammonium vanadate (V).

**Ammonium vanadate (V)**  $\text{NH}_4\text{VO}_3$  [K 8747, Special class]

**Amosulalol hydrochloride for assay**  $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_5\text{S} \cdot \text{HCl}$  [Same as the monograph Amosulalol Hydrochloride. It contains not less than 99.0% of amosulalol hydrochloride ( $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_5\text{S} \cdot \text{HCl}$ ), calculated on the anhydrous basis.]

**Amoxicillin** See amoxicillin hydrate.

**Amoxicillin hydrate**  $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S} \cdot 3\text{H}_2\text{O}$  [Same as the namesake monograph]

**Amphoteric electrolyte solution for pH 3 to 10** Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 3 to 10 when mixed with polyacrylamide gel and placed in an electric field.

**Amphoteric electrolyte solution for pH 6 to 9** Forms a pH gradient over a pH range of 6 to 9 when mixed with polyacrylamide gel and placed in an electric field. Prepare by diluting a 0.35 mmol/pH-mL buffer capacity solution about 20-fold with water. Almost colorless.

**Amphoteric electrolyte solution for pH 8 to 10.5** Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 8 to 10.5 when mixed with polyacrylamide gel and placed in an electric field.

**Amygdalin for assay** Amygdalin for thin-layer chromatography. However, it meets the following requirements:

**Absorbance**  $\langle 2.24 \rangle E_{1\text{cm}}^{1\%}$  (263 nm): 5.2–5.8 [20 mg, methanol, 20 mL; separately determine the water  $\langle 2.48 \rangle$  (5 mg, coulometric titration) and calculate on the anhydrous basis].

**Purity** Related substances—Dissolve 5 mg of amygdalin for assay in 10 mL of the mobile phase, and use this as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography  $\langle 2.01 \rangle$  according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than amygdalin from the sample solution is not larger than the peak area of amygdalin from the standard solution. Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Keishibukuryogan Extract.

Time span of measurement: About 3 times as long as the retention time of amygdalin.

System suitability

Test for required detectability: Pipet 1 mL of the standard

solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of amygdalin obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Keishibukuryogan Extract.

**Amygdalin for component determination** See amygdalin for assay.

**Amygdalin for thin-layer chromatography**  $C_{20}H_{27}NO_{11}$  A white, odorless powder. Soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

**Identification**—Determine the absorption spectrum of a solution of amygdalin for thin-layer chromatography in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, between 261 nm and 265 nm, and between 267 nm and 271 nm.

**Purity** Related substances—Dissolve 5 mg of amygdalin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Peach Kernel: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

***n*-Amyl alcohol**  $CH_3(CH_2)_4OH$  Clear, colorless liquid, having a characteristic odor. Sparingly soluble in water, and miscible with ethanol (95) and with diethyl ether.

**Refractive index** <2.45>  $n_D^{20}$ : 1.409 – 1.411

**Specific gravity** <2.56>  $d_4^{20}$ : 0.810 – 0.820

**Distilling range** <2.57>: 135 – 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 and with 2-butanone, and freely soluble in water.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.808 – 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 <2.50> 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** <2.57>: 100 – 103°C, not less than 95 vol%.

***tert*-Amyl alcohol** See *t*-amyl alcohol.

**Amyl alcohol, iso** See 3-methyl-1-butanol.

**Anesthetic ether** See ether, anesthetic.

**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 hydrazine for amino acid analysis** Prepared for amino acid analysis.

**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.

**Anhydrous sodium sulfite** See sodium sulfite, anhydrous.

**Aniline**  $C_6H_5NH_2$  [K 8042, Special class]

**Animal tissue peptone** See peptone, animal tissue.

***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.

**Anisole**  $C_7H_8O$  A colorless liquid. Boiling point: about 155°C.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.995 – 1.001.

**14-Anisoylaconine hydrochloride for assay**  $C_{33}H_{47}NO_{11} \cdot HCl \cdot xH_2O$  White crystalline powder or powder. Freely soluble in methanol, sparingly soluble in water and in ethanol (99.5). Melting point: about 210°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (258 nm): 276 – 294 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** (1) Related substances—To 1.0 mg of 14-anisoylaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principle spot at around *R<sub>f</sub>* value of 0.5 appears.

(2) Related substances—Dissolve 5.0 mg of 14-anisoylaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 14-anisoylaconine obtained from the sample solution is not larger than the peak area of 14-anisoylaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 4 times as long as the retention time of 14-anisoylaconine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of 14-anisoylaconine obtained from 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of 14-anisoylaconine from 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

**14-Anisoylaconine hydrochloride for component determination** See 14-anisoylaconine hydrochloride for assay.

**Anode solution A for water determination** Dissolve 100 g of diethanolamine in 900 mL of a mixture of methanol for water determination and chloroform for water determination (1:1), pass dried sulfur dioxide gas through this solution while cooling until the mass increase of the solution reaches 64 g. Then add 20 g of iodine, and add water until the color of the solution changes from brown to yellow. To 600 mL of this solution add 400 mL of chloroform for water determination.

**Anthrone**  $C_{14}H_{10}O$  Light yellow crystals or crystalline powder.

*Melting point* <2.60>: 154 – 160°C.

Preserve in a light-resistant tight container.

**Anthrone TS** Dissolve 35 mg of anthrone in 100 mL of sulfuric acid.

**Anti-A type antibody for blood typing** Conforms to the requirements of antibody for blood typing.

**Anti-B type antibody for blood typing** Conforms to the requirements of antibody for blood typing.

**Antibody fragment (Fab')** Purify E. coli protein antibody by affinity chromatography using *Staphylococcus aureus* protein A as a ligand, and fractionate IgG. Digest this fraction using pepsin, remove the pepsin and Fc fragment by gel filtration chromatography, and obtain F(ab)<sub>2</sub> fragment after removing undigested IgG by affinity chromatography with protein A as ligand. Reduce this with 2-mercaptoethylamine.

**Anti-bradykinin antibody** A colorless to light brown, clear solution prepared by dissolving rabbit origin anti-bradykinin antibody in 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL of bovine serum albumin.

**Performance test**—To a suitable amount of anti-bradykinin antibody to be tested add 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL bovine serum albumin to make a 1 vol% solution. Perform the test with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the absorbances at 490 – 492 nm,  $A_1$  and  $A_2$ , of the standard solution (1) and the standard solution (7): the value,  $A_2 - A_1$ , is not less than 1.

**Anti-bradykinin antibody TS** To 0.15 mL of anti-bradykinin antibody, 15 mg of bovine serum albumin, 2.97 mg of sodium dihydrogen phosphate dihydrate, 13.5 mg of disodium hydrogen phosphate dodecahydrate and 13.5 mg of sodium chloride add water to make 15 mL, and lyophilize. Dissolve this in 15 mL of water. Prepare before use.

**Anti-E. coli protein antibody stock solution** Taking E. coli protein stock solution as the immunogen, mix with Freund's complete adjuvant, and immunize rabbits by subcutaneous injection at 3 week intervals to obtain antiserum. Treat the antiserum obtained by ammonium sulfate precipitation.

Protein concentration: Dilute anti-E. coli protein antibody stock solution with 0.05 mol/L tris hydrochloride buffer solution (pH 7.5), measure the absorbance at 280 nm using 0.05 mol/L tris hydrochloride buffer solution (pH 7.5) as a control as direct under Ultraviolet-visible Spectrophotometry <2.24>, and determine the protein concentration (absorbance 1.0 = 0.676 mg/mL).

**Antimony (III) chloride**  $SbCl_3$  [K 8400, 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 (III) chloride to make a saturated solution, and place in light-resistant, glass-stoppered bottles. Prepare before use.

**Antimony trichlorid** See antimony (III) chloride.

**Antimony trichlorid TS** See antimony (III) chlorid TS.

**Antipyrene**  $C_{11}H_{12}N_2O$  [Same as the namesake monograph]

**Anti-rabbit antibody-coated wells** Wells of a polystyrene microplate coated with goat origin anti-rabbit IgG antibody.

**Anti-thrombin III** A white powder.

*Water* <2.48>: not more than 5%.

*Content*: not less than 80% and not more than 130% of the labeled amount.

**Anti-thrombin III TS** Dissolve 10 unit of anti-thrombin III in 10 mL of water.

**Anti-ulinastatin rabbit serum** To a suitable amount of Ulinastatin having the specific activity of more than 3000 Units per mg protein add isotonic sodium chloride solution so that each mL of the solution contains about 1 mg of protein. To 1 mL of this solution add 1 mL of Freund's complete adjuvant, and emulsify completely. Intracutaneously, inject the emulsion so obtained into a rabbit weighing about

2 kg. Repeat the injection at least 4 times at one-week intervals, and draw the blood of the animal from the carotid artery after the antibody titer reaches 16 times or more. Separate the serum after the blood has coagulated. Preserve at below  $-20^{\circ}\text{C}$ .

**Anti-urokinase serum** Take Urokinase containing not less than 140,000 Unit per mg of protein, dissolve in isotonic sodium chloride solution to make a solution containing 1 mg of protein per mL, and emulsify with an equal volume of Freund's complete adjuvant. Inject intracutaneously three 2-mL portions of the emulsion to a healthy rabbit weighed between 2.5 kg and 3.0 kg in a week interval. Collect the blood from the rabbit at 7 to 10 days after the last injection, and prepare the anti-serum.

**Performance test**—Dissolve 1.0 g of agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish to make a depth of about 2 mm. After cooling, bore three of a pair-well 2.5 mm in diameter with a space of 6 mm each other. In one of the wells of each pair-well, place 10  $\mu\text{L}$  of anti-urokinase serum, and in each another well, place 10  $\mu\text{L}$  of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution, 10  $\mu\text{L}$  of human serum and 10  $\mu\text{L}$  of human urine, respectively, and allow to stand overnight: a precipitin line appears between anti-urokinase serum and urokinase, and not appears between anti-urokinase serum and human serum or human urine.

**$\alpha$ -Apoxytetracycline**  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_8$  Yellow-brown to green powder.

**Melting point** <2.60>:  $200 - 205^{\circ}\text{C}$

**$\beta$ -Apoxytetracycline**  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_8$  Yellow-brown to brown powder.

**Purity** Related substances—Dissolve 8 mg of  $\beta$ -apoxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Proceed the test with 20  $\mu\text{L}$  of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than  $\beta$ -apoxytetracycline is not more than 10%.

**Aprindine hydrochloride for assay**  $\text{C}_{22}\text{H}_{30}\text{N}_2\cdot\text{HCl}$   
[Same as the monograph Aprindine Hydrochloride. When dried, it contains not less than 99.5% of aprindine hydrochloride ( $\text{C}_{22}\text{H}_{30}\text{N}_2\cdot\text{HCl}$ ).]

**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 15,000 KIE Units and not more than 25,000 KIE Units of aprotinin per mL. **Assay**—(i) Trypsin solution: Weigh an amount of crystalline 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 10 mL. Prepare before use, and preserve in ice. (ii) Sample solution: Dilute a suitable quantity of aprotinin with sodium tetraborate-calcium chloride buffer solution, pH 8.0 so that each mL 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 reac-

tion reservoir, 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver-silver chloride electrode, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at  $25 \pm 0.1^{\circ}\text{C}$  by means of a precise thermoregulator. (iv) Procedure: To 5.0 mL of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, and use this solution as the substrate solution. Pipet 1 mL 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 1 mL of the test solution I previously allowed to stand at  $25 \pm 0.1^{\circ}\text{C}$  for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50- $\mu\text{L}$  micropipet (minimum graduation of 1  $\mu\text{L}$ ), while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2 mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution 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 exactly 1 mL of the test solution II, previously allowed to stand at  $25 \pm 0.1^{\circ}\text{C}$  for 10 minutes, and proceed in the same manner. Separately, 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 1 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, previously allowed to stand at  $25 \pm 0.1^{\circ}\text{C}$  for 10 minutes, and perform a blank determination in the same manner. (v) Calculation: Plot the amount of consumption ( $\mu\text{L}$ ) of 0.1 mol/L sodium 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  $\mu\text{mol}$  of sodium hydroxide consumed per minute as  $D$ .

$$D (\mu\text{mol NaOH}/\text{min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10} \times f$$

$f$ : Factor of 0.1 mol/L sodium hydroxide VS

KIE Units per mL of aprotinin to be tested

$$= \frac{2(D_A - D_0) - (D_B - D_0)}{L} \times n \times 32.5$$

$L$ : Amount (mL) of the sample solution added to the test solution II

$n$ : Dilution coefficient of aprotinin to be tested

$D_A$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the test solution I is used

$D_B$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the test solution II is used

$D_0$ :  $\mu\text{mol}$  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 kallidinogenase 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 per mL.

**Aqua regia** Add 1 volume of nitric acid to 3 volumes of hydrochloric acid. Prepare before use.

**L-Arabinose**  $C_5H_{10}O_5$  A white crystalline powder.

**Melting point** <2.60>: 155 – 160°C.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +103.0 – +105.5° Weigh accurately about 5 g of L-arabinose, previously dried at 105°C for 2 hours, dissolve in 30 mL of water, add 0.4 mL of ammonia TS, and add water to make exactly 50 mL. Allow to stand for 1 hour, and determine using a 100-mm cell.

**Arbutin for assay** Use arbutin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (280 nm): 70 – 76 [4 mg, previously dried in a desiccator (in vacuum, silica gel), 12 hours, water, 100 mL].

**Purity** Related substances—Dissolve 40 mg of arbutin for assay in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than that of arbutin from the sample solution is not larger than the peak area of arbutin from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Assay under Bearberry Leaf except detection sensitivity and time span of measurement.

**Detection sensitivity:** Pipet 1 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of arbutin obtained from 10  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method and the peak height of arbutin obtained from 10  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About 3 times as long as the retention time of arbutin beginning after the solvent peak.

**Arbutin for component determination** See arbutin for assay.

**Arbutin for thin-layer chromatography**  $C_{12}H_{16}O_7 \cdot nH_2O$  Colorless to white crystals or crystalline powder, and odorless. Freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ethyl acetate and in chloroform.

**Melting point** <2.60>: 199 – 201°C

**Purity** Related substances—Dissolve 1.0 mg of arbutin for thin-layer chromatography in exactly 1 mL of a mixture

of ethanol (95) and water (7:3). Perform the test with 20  $\mu\text{L}$  of this solution as directed in the Identification (2) under Bearberry Leaf: any spot other than the main spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Arecoline hydrobromide for thin-layer chromatography**  $C_8H_{13}NO_2 \cdot HBr$  White crystals. Freely soluble in water, soluble in methanol, and practically insoluble in diethyl ether.

**Melting point** <2.60>: 169 – 171°C

**Purity** Related substances—Dissolve 5 mg of arecoline hydrobromide for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Areca: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.6 does not appear.

**L-Arginine**  $C_6H_{14}N_4O_2$  White, crystals or crystalline powder. It has a characteristic odor.

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +26.9 – +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50 mL, 200 mm).

**Loss on drying** <2.41>: not more than 0.50% (1 g, 105°C, 3 hours).

**Content:** not less than 98.0% and not more than 102.0%. **Assay**—Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <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.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 8.710 \text{ mg of } C_6H_{14}N_4O_2 \end{aligned}$$

**L-Arginine hydrochloride**  $C_6H_{14}N_4O_2 \cdot HCl$  [Same as the namesake monograph]

**Aristolochic acid I for crude drugs purity test**

$C_{17}H_{11}NO_7$  Yellow crystalline powder. Melting point: about 280°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (318 nm): 384 – 424 (1 mg, methanol, 100 mL).

**Purity** Related substances—Dissolve 1.0 mg of aristolochic acid I for crude drugs purity test in 100 mL of diluted methanol (3 in 4), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aristolochic acid I obtained from the sample solution is not larger than the peak area of aristolochic acid I from the standard solution.

**Operating conditions**

**Detector, column, column temperature, mobile phase, and flow rate:** Proceed as directed in the operating conditions in the Purity (4) under Asiasarum Root.

**Time span of measurement:** About 3 times as long as the retention time of aristolochic acid I beginning after the solvent peak.

**System suitability**

Proceed as directed in the system suitability in the Purity

(4) under Asiasarum Root.

**Arsenazo III**  $C_{22}H_{18}As_2N_4O_{14}S_2$  [K 9524, Special class]

**Arsenazo III TS** Dissolve 0.1 g of arsenazo III in water to make 50 mL.

**Arsenic-free zinc** See zinc for arsenic analysis.

**Arsenic (III) trioxide**  $As_2O_3$  [K 8044, Arsenic (III) trioxide, Special class]

**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.

**Arsenic trioxide** See arsenic (III) trioxide.

**Arsenic trioxide TS** See arsenic (III) trioxide TS.

**Asarinin for thin-layer chromatography**  $C_{20}H_{18}O_6$   
White crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. *Melting point*: 118 – 122°C.

*Identification*—Determine the absorption spectrum of a solution in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 285 nm and 289 nm.

*Purity* Related substances—Dissolve 1 mg in 1 mL of methanol, and perform the test with 1  $\mu$ L of this solution as directed in the Identification (7) under Shoseiryuto Extract: no spot other than the principal spot (*Rf* value is about 0.4) appears.

**(E)-Asarone**  $C_{12}H_{16}O_3$  White powder. Freely soluble in methanol and in ethanol (99.5) and practically insoluble in water. *Melting point*: about 60°C.

*Identification*—Determine the infrared absorption spectrum of (*E*)-asarone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, it exhibits absorption at the wave numbers of about 2990  $cm^{-1}$ , 2940  $cm^{-1}$ , 2830  $cm^{-1}$ , 1609  $cm^{-1}$ , 1519  $cm^{-1}$ , 1469  $cm^{-1}$ , 1203  $cm^{-1}$ , 1030  $cm^{-1}$ , 970  $cm^{-1}$  and 860  $cm^{-1}$ .

*Purity* Related substances—Dissolve 2 mg of (*E*)-asarone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 (*E*)-asarone obtained from the sample solution is not larger than the peak area of (*E*)-asarone from the standard solution.

*Operating conditions*

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Perilla Herb.

Time span of measurement: About 3 times as long as the retention time of (*E*)-asarone beginning after the solvent peak.

System suitability

System performance: Proceed as directed in the system suitability in the Assay under Perilla Herb.

**Ascorbic acid** See L-ascorbic acid.

**L-Ascorbic acid**  $C_6H_8O_6$  [K 9502, L(+)-Ascorbic Acid, Special class]

**Ascorbic acid for iron limit test** See L-ascorbic acid.

**0.012 g/dL L-Ascorbic acid-hydrochloric acid TS** Dissolve 15 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

**0.02 g/dL L-Ascorbic acid-hydrochloric acid TS** Dissolve 25 mg 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 L-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.

**DL-Aspartic acid**  $C_4H_7NO_4$  A white crystalline powder that is sparingly soluble in water. *Melting point*: 270 to 271°C.

**L-Aspartic acid**  $C_4H_7NO_4$  [K 9045, Special class]

**Aspartic acid** See L-aspartic acid.

**Aspirin**  $C_9H_8O_4$  [Same as the namesake monograph]

**Astragaloside IV for thin-layer chromatography**  $C_{41}H_{68}O_{14}$  A white powder. Sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +19 – +26° (10 mg dried with silica gel for 24 hours, methanol, 2 mL, 50 mm).

*Purity* Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 5  $\mu$ L of the standard solution as directed in the Identification (4) under Hochuekkito Extract: no spot appears other than the principal spot of around *Rf* 0.4.

**Atractylenolide III for thin-layer chromatography**  $C_{15}H_{20}O_3$  White crystals or crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water. *Melting point*: 193 – 196°C

*Identification*—Determine the absorption spectrum of a solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 217 nm and 221 nm.

*Purity* Related substances—Dissolve 1 mg in 10 mL of methanol. Proceed the test with 2  $\mu$ L of the standard solution as directed in the Identification (3) under Kamishoyosan Extract: no spot appears other than the principal spot of around *Rf* 0.5

**Atropine sulfate** See atropine sulfate hydrate.

**Atropine sulfate for assay** See atropine sulfate hydrate for assay.

**Atropine sulfate for thin-layer chromatography** See atropine sulfate hydrate for thin-layer chromatography.

**Atropine sulfate hydrate**  $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$  [Same as the namesake monograph]

**Atropine sulfate hydrate for assay**  $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$  [Same as the monograph Atropine Sulfate Hydrate. When dried, it contains not less than 99.0% of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4]$ .]

**Atropine sulfate hydrate for thin-layer chromatography** ( $C_{17}H_{23}NO_3$ )<sub>2</sub>·H<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O Use atropine sulfate hydrate for assay meeting the following additional specifications. Weigh accurately about 50 mg of atropine sulfate hydrate for assay, dissolve in ethanol (95) to make 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 50  $\mu$ L of the solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and diethylamine (9:1) to a distance of about 10 cm, air-dry the plate, and spray evenly chloroplatinic acid-potassium iodide TS on the plate: any spot other than the spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**A-type erythrocyte suspension** Prepare a suspension containing 1 vol% of erythrocyte separated from human A-type blood in isotonic sodium chloride solution.

**Azelastine hydrochloride for assay**  $C_{22}H_{24}ClN_3O$ ·HCl [Same as the monograph Azelastine Hydrochloride]

**Baicalin for thin-layer chromatography** See baicalin hydrate for thin-layer chromatography.

**Baicalin hydrate for thin-layer chromatography**  $C_{21}H_{18}O_{11}$ ·H<sub>2</sub>O Light yellow odorless powder. Slightly soluble in methanol, and practically insoluble in water and in diethyl ether. Melting point: about 206°C (with decomposition).

**Purity** Related substance—Dissolve 1.0 mg of baicalin hydrate for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification (2) under Scutellaria Root: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Bakumondo** [Same as the namesake monograph]

**Balsam** Canada balsam for microscopy. Before use, dilute to a suitable concentration with xylene.

**Bamethan sulfate** ( $C_{12}H_{19}NO_2$ )<sub>2</sub>·H<sub>2</sub>SO<sub>4</sub> [Same as the namesake monograph]

**Barbaloin for assay**  $C_{21}H_{22}O_9$  Use barbaloin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (360 nm): 260 – 290 [10 mg dried in a desiccator (in vacuum, phosphorus (V) oxide) for not less than 24 hours, methanol, 500 mL.]

**Purity** Related substances—Dissolve 10 mg of the substance to be tested in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than barbaloin from the sample solution is not larger than the peak area of barbaloin from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Assay under Aloe except wavelength, detection sensitivity and time span of measurement.

**Wavelength:** 300 nm

**Detection sensitivity:** Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of barbaloin obtained from 20  $\mu$ L of the standard solution (2) can be measured by the automatic integration method and the peak height of barbaloin obtained from 20  $\mu$ L of the standard solution (1) shows about 20% of the full scale.

**Time span of measurement:** About 3 times as long as the retention time of barbaloin beginning after the solvent peak.

**Barbaloin for component determination** See barbaloin for assay.

**Barbaloin for thin-layer chromatography**  $C_{21}H_{22}O_9$  Light yellow, crystalline powder. Freely soluble in methanol, practically insoluble in water and in diethyl ether.

**Melting point** <2.60>: 148°C

**Purity** Related substances—Dissolve 1.0 mg of barbaloin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification (2) under Aloe: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.6 does not appear.

**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** <2.54>—The pH of a solution of barbital sodium (1 in 200) is between 9.9 and 10.3.

**Loss on drying** <2.41>: 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 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract with three 25-mL portions of chloroform, combine the total extract, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5-mL portions of chloroform, combine the filtrate and the washings, add 10 mL of ethanol (95), and titrate <2.50> 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.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 20.62 mg of  $C_8H_{11}N_2NaO_3$

**Barium chloride** See barium chloride dihydrate.

**Barium chloride dihydrate**  $BaCl_2 \cdot 2H_2O$  [K 8155, Special class]

**Barium chloride TS** Dissolve 12 g of barium chloride dihydrate in water to make 100 mL (0.5 mol/L).

**Barium hydroxide** See barium hydroxide octahydrate.

**Barium hydroxide octahydrate**  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$   
[K 8577, 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**  $\text{Ba}(\text{NO}_3)_2$  [K 8565, Special class]

**Barium nitrate TS** Dissolve 6.5 g of barium nitrate in water to make 100 mL (0.25 mol/L).

**Barium oxide**  $\text{BaO}$  A white to yellowish or grayish white, powder.

*Identification* (1) Dissolve 0.5 g in 15 mL of water and 5 mL of hydrochloric acid, and add 10 mL of dilute sulfuric acid: white precipitates appear.

(2) Perform the test as directed under Flame Coloration Test (1) <1.04>: a green color appears.

**Barium perchlorate**  $\text{Ba}(\text{ClO}_4)_2$  [K 9551, Special class]

**Becanamycin sulfate**  $\text{C}_{18}\text{H}_{37}\text{N}_5\text{O}_{10} \cdot x\text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Beclometasone dipropionate**  $\text{C}_{28}\text{H}_{37}\text{ClO}_7$  [Same as the namesake monograph]

**Benidipine hydrochloride**  $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$  [Same as the namesake monograph]

**Benidipine hydrochloride for assay**  $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$   
[Same as the monograph Benidipine Hydrochloride. When dried, it contains not less than 99.5% of benidipine hydrochloride ( $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$ )]

**Benzaldehyde**  $\text{C}_6\text{H}_5\text{CHO}$  [K 8857, First class]

**Benzalkonium chloride** [Same as the namesake monograph]

**Benzalophthalide**  $\text{C}_{15}\text{H}_{10}\text{O}_2$  Yellow crystalline powder. Melting point: 99 – 102°C

**Benz[a]anthracene**  $\text{C}_{18}\text{H}_{12}$  White to yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 158 – 163°C.

*Identification* Perform the test with benz[a]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  228) and a fragment ion peak ( $m/z$  114).

*Purity* Related substances—Dissolve 3.0 mg of benz[a]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1  $\mu\text{L}$  of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benz[a]anthracene is not more than 2.0%.

Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5  $\mu\text{m}$ .

Column temperature: Inject at a constant temperature of

about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: At a constant temperature of about 250°C.

Interface temperature: At a constant temperature of 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benz[a]anthracene is about 15 minutes.

Split ratio: Splitless.

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of benz[a]anthracene obtained from 1  $\mu\text{L}$  of this solution is equivalent to 5 to 15% of that of benz[a]anthracene from 1  $\mu\text{L}$  of the standard solution.

**Benzene**  $\text{C}_6\text{H}_6$  [K 8858, Special class]

**Benzethonium chloride for assay**  $\text{C}_{27}\text{H}_{42}\text{ClNO}_2$  [Same as the monograph Benzethonium Chloride. When dried, it contains not less than 99.0% of benzethonium chloride ( $\text{C}_{27}\text{H}_{42}\text{ClNO}_2$ ).]

**Benzoic acid**  $\text{C}_6\text{H}_5\text{COOH}$  [K 8073, Special class]

**Benzoin**  $\text{C}_6\text{H}_5\text{CH}(\text{OH})\text{COC}_6\text{H}_5$  White to pale yellow, crystals or powder.

*Melting point* <2.60>: 132 – 137°C

**Benzophenone**  $\text{C}_6\text{H}_5\text{COC}_6\text{H}_5$  Colorless crystals, having a characteristic odor.

*Melting point* <2.60>: 48 – 50°C

**Benzo[a]pyrene**  $\text{C}_{20}\text{H}_{12}$  Light yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 176 – 181°C.

*Identification*—Perform the test with benzo[a]pyrene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  252) and a fragment ion peak ( $m/z$  125).

*Purity* Related substances—Dissolve 3.0 mg of benzo[a]pyrene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1  $\mu\text{L}$  of this solution as directed under Gas Chromatography <2.02> under the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benzo[a]pyrene is not more than 3.0%.

Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25–0.5  $\mu\text{m}$ .

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: A constant temperature of about 250°C.

Interface temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo[*a*]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL. Confirm that the peak area of benzo[*a*]pyrene obtained from 1  $\mu$ L of this solution is equivalent to 5 to 15% of that of benzo[*a*]pyrene from 1  $\mu$ L of the sample solution.

***p*-Benzoquinone** C<sub>6</sub>H<sub>4</sub>O<sub>2</sub> Yellow to yellow-brown, crystals or crystalline powder, having a pungent odor. Soluble in ethanol (95) and in diethyl ether, slightly soluble in water. It is gradually changed to a blackish brown color by light.

*Melting point* <2.60>: 111 – 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 25 mL of water and 25 mL of diluted sulfuric acid (1 in 15), dissolve 3 g of potassium iodide by shaking, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 5.405 mg of C<sub>6</sub>H<sub>4</sub>O<sub>2</sub>

***p*-Benzoquinone TS** Dissolve 1 g of *p*-benzoquinone in 5 mL of acetic acid (100), and add ethanol (95) to make 100 mL.

***N*- $\alpha$ -Benzoyl-L-arginine ethyl ester hydrochloride**

C<sub>15</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>·HCl White crystals or crystalline powder. Freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

*Melting point* <2.60>: 129 – 133°C

*Optical rotation* <2.49> [ $\alpha$ ]<sub>D</sub><sup>20</sup>: –15.5 – –17.0° (2.5 g, water, 50 mL, 100 mm).

*Purity* (1) Clarity and color of solution—Dissolve 0.1 g of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Related substances—Weigh 0.10 g of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 6 mL of water, add 4 mL of hydrochloric acid, heat in a boiling water bath for 5 minutes to decompose, and use this solution as the sample solution. Perform the test with the sample solution as directed under Paper Chromatography. Spot 5  $\mu$ L of the sample solution on a chromatographic filter paper. Develop with a mixture of water, acetic acid (100) and 1-butanol (5:4:1) to a distance of about 30 cm, and air-dry the paper. Spray evenly a solution of ninhydrin in acetone (1 in 50) upon the paper, and heat at 90°C for 10 minutes: only one purple spot appears.

*Content*: not less than 99.0%. *Assay*—Weigh accurately about 0.6 g of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 50 mL of water, neutralize with 0.1 mol/L sodium hydroxide VS, if necessary, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 4 drops of dichlorofluorescein TS).

Each mL of 0.1 mol/L silver nitrate VS  
= 34.28 mg of C<sub>15</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>·HCl

***N*- $\alpha$ -Benzoyl-L-arginine ethyl ester TS** Dissolve 0.07 g of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride in freshly boiled and cooled water to make exactly 10 mL.

***N*- $\alpha$ -Benzoyl-L-arginine-4-nitroanilide hydrochloride**

C<sub>19</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>·HCl Light yellow crystalline powder.

*Optical rotation* <2.49> [ $\alpha$ ]<sub>D</sub><sup>20</sup>: +45.5 – +48.0° (after drying, 0.5 g, *N,N*-dimethylformamide, 25 mL, 100 mm).

*Purity* Related substances—Dissolve 0.20 g of *N*- $\alpha$ -benzoyl-L-arginine-4-nitroanilide hydrochloride in 10 mL of *N,N*-dimethylformamide, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Exposure the plate to a vapor of iodine: only one spot appears.

***N*- $\alpha$ -Benzoyl-L-arginine-4-nitroanilide TS** Dissolve 0.1 g of *N*- $\alpha$ -benzoyl-L-arginine-4-nitroanilide hydrochloride in water to make 100 mL.

**Benzoyl chloride** C<sub>6</sub>H<sub>5</sub>COCl A clear and colorless fuming liquid. Specific gravity: about 1.2 g/mL.

*Identification*—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1775 cm<sup>-1</sup>, 1596 cm<sup>-1</sup>, 1450 cm<sup>-1</sup>, 1307 cm<sup>-1</sup>, 1206 cm<sup>-1</sup>, 873 cm<sup>-1</sup>, 776 cm<sup>-1</sup> and 671 cm<sup>-1</sup>.

**Benzoylhypaconine hydrochloride for assay**

C<sub>31</sub>H<sub>43</sub>NO<sub>9</sub>·HCl·xH<sub>2</sub>O White crystals or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5). Melting point: about 230°C (with decomposition).

*Absorbance* <2.24> E<sub>1</sub><sup>1%</sup><sub>cm</sub> (230 nm): 225 – 240 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

*Purity* (1) Related substances—To 1.0 mg of benzoylhypaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around R<sub>f</sub> value of 0.5 appears.

(2) Related substance—Dissolve 5.0 mg of benzoylhypaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 benzoylhypaconine obtained from the sample solution is not larger than the peak area of benzoylhypaconine from the standard solution.

*Operating conditions*

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 5 times as long as the retention time of benzoylhypaconine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylhypaconine obtained from 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of benzoylhypaconine from 20  $\mu$ L of the standard solution.

System performance: When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

**Benzoylhypaconine hydrochloride for component determination** See benzoylhypaconine hydrochloride for assay.

**N-Benzoyl-L-isoleucyl-L-glutamyl( $\gamma$ -OR)-glycyl-L-arginyl-p-nitroanilide hydrochloride** An equal amount mixture of two components, R = H and R = CH<sub>3</sub>. A white powder. Slightly soluble in water.

Absorbance <2.24>  $E_{1\text{cm}}^{1\%}$  (316 nm): 166 – 184 (10 mg, water, 300 mL).

**Benzoylmesaconine hydrochloride for assay** Benzoylmesaconine hydrochloride for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 5.0 mg of benzoylmesaconine hydrochloride for assay in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 benzoylmesaconine obtained from the sample solution is not larger than the peak area of benzoylmesaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 6 times as long as the retention time of benzoylmesaconine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of benzoylmesaconine obtained from 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of benzoylmesaconine from 20  $\mu$ L of the standard solu-

tion.

System performance: When the procedure is run with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

**Benzoylmesaconine hydrochloride for component determination** See benzoylmesaconine hydrochloride for assay.

**Benzoylmesaconine hydrochloride for thin-layer chromatography** C<sub>31</sub>H<sub>43</sub>NO<sub>10</sub>·HCl·xH<sub>2</sub>O White crystals or crystalline powder. Soluble in water and in ethanol (99.5) and sparingly soluble in methanol. Melting point: about 250°C (with decomposition).

Absorbance <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 217 – 231 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

**Purity** Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 1 mL of ethanol (99.5). Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around R<sub>f</sub> value of 0.4 appears.

**Benzoyl peroxide, 25% water containing** (C<sub>6</sub>H<sub>5</sub>CO)<sub>2</sub>O<sub>2</sub> White moist crystals or powder. Soluble in diethyl ether and in chloroform, and very slightly soluble in water and in ethanol (95). Melting point: 103 – 106°C (dried substance) (with decomposition).

Loss on drying <2.41>: not more than 30% (0.1 g, in vacuum, silica gel, constant mass).

**Benzyl alcohol** C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>OH Clear and colorless liquid, having a characteristic odor.

Specific gravity <2.56>  $d_{20}^{20}$ : 1.045 – 1.050.

Preserve in a light-resistant tight container.

**Benzyl benzoate** C<sub>6</sub>H<sub>5</sub>COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> A colorless oily liquid. Congealing point: about 18°C. Boiling point: about 323°C.

Specific gravity <2.56>  $d_{20}^{20}$ : 1.118 – 1.123.

Preserve in a light-resistant tight container.

**Benzyl parahydroxybenzoate** HOC<sub>6</sub>H<sub>4</sub>COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> White, odorless, fine crystals or crystalline powder. Freely soluble in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

Melting point <2.60>: 109 – 112°C

Residue on ignition <2.44>: not more than 0.1%.

Content: not less than 99.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS  
= 228.2 mg of C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>

**Benzylpenicillin benzathin** See benzylpenicillin benzathine hydrate.

**Benzylpenicillin benzathine hydrate**  
(C<sub>16</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S)<sub>2</sub>·C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>·4H<sub>2</sub>O [Same as the namesake

monograph]

**Benzylpenicillin potassium**  $C_{16}H_{17}KN_2O_4S$  [Same as the monograph Benzylpenicillin Potassium]

**Benzyl *p*-hydroxybenzoate** See benzyl parahydroxybenzoate.

***p*-Benzylphenol**  $C_6H_5CH_2C_6H_4OH$  White to pale yellowish white crystals or crystalline powder.

*Melting point* <2.60>: 80 – 85°C

**Beraprost sodium**  $C_{24}H_{29}NaO_5$  [Same as the namesake monograph]

**Beraprost sodium for assay**  $C_{24}H_{29}NaO_5$  [Same as the monograph Beraprost Sodium. When dried it contains not less than 99.0% of beraprost sodium ( $C_{24}H_{29}NaO_5$ ).]

**Berberine chloride** See berberin chloride hydrate.

**Berberine chloride hydrate**  $C_{20}H_{18}ClNO_4 \cdot xH_2O$  [Same as the namesake monograph]

**Berberine chloride for thin-layer chromatography** See berberine chloride hydrate for thin-layer chromatography.

**Berberine chloride hydrate for thin-layer chromatography** [Same as the monograph Berberine Chloride Hydrate. Use the berberine chloride meeting the following additional specifications.]

*Purity* Related substances—Dissolve 10 mg of berberine chloride for thin-layer chromatography in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Phellodendron Bark: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Bergenin for thin-layer chromatography**  $C_{14}H_{16}O_9 \cdot xH_2O$  White crystals or crystalline powder. Slightly soluble in ethanol (99.5), very slightly soluble in water, and practically insoluble in diethyl ether.

*Identification*—Determine the absorption spectrum of a solution of bergenin for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 217 nm and 221 nm, and between 273 nm and 277 nm, and a minimum between 241 nm and 245 nm.

*Purity* Related substances—Dissolve 1.0 mg of bergenin for thin-layer chromatography in 1 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification under Mallotus Bark: no spot other than the principal spot at the *R<sub>f</sub>* value of about 0.5 appears.

**Betahistine mesilate**  $C_8H_{12}N_2 \cdot 2CH_4O_3S$  [Same as the namesake monograph]

**Betahistine mesilate for assay** [Same as the monograph Betahistine Mesilate. When dried, it contains not less than 99.0% of betahistine mesilate ( $C_8H_{12}N_2 \cdot 2CH_4O_3S$ ).]

**Bezafibrate for assay**  $C_{19}H_{20}ClNO_4$  [Same as the monograph Bezafibrate. When dried it contains not less than 99.0% of bezafibrate ( $C_{19}H_{20}ClNO_4$ ).]

**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 portions 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.

**$\alpha$ -BHC ( $\alpha$ -Hexachlorocyclohexane)**  $C_6H_6Cl_6$

*Melting point* <2.60>: 157 – 159°C

*Purity* Related substances—Dissolve 10 mg of  $\alpha$ -BHC in 5 mL of acetone for purity of crude drug, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu$ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than  $\alpha$ -BHC from the sample solution is not larger than the peak area of  $\alpha$ -BHC from the standard solution (1).

*Operating conditions*

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

*Detection sensitivity*: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make 20 mL, and use this solution as standard solution (2). Adjust the detection sensitivity so that the peak area of  $\alpha$ -BHC obtained from 1 mL of the standard solution (2) can be measured by the automatic integration method, and the peak height of  $\alpha$ -BHC from 1  $\mu$ L of the standard solution (1) is about 20% of the full scale.

*Time span of measurement*: About twice as long as the retention time of  $\alpha$ -BHC beginning after the peak of solvent.

**$\beta$ -BHC ( $\beta$ -Hexachlorocyclohexane)**  $C_6H_6Cl_6$

*Melting point* <2.60>: 308 – 310°C

*Purity* Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC using the following standard solution (1).

Standard solution (1): Pipet 2 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**$\gamma$ -BHC ( $\gamma$ -Hexachlorocyclohexane)**  $C_6H_6Cl_6$

*Melting point* <2.60>: 112 – 114°C

*Purity* Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC.

**$\delta$ -BHC ( $\delta$ -Hexachlorocyclohexane)**  $C_6H_6Cl_6$

*Melting point* <2.60>: 137 – 140°C

*Purity* Related substances—Proceed as directed in the Purity under  $\alpha$ -BHC using the following standard solution (1).

Standard solution (1): Pipet 5 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100

mL.

**Bifonazole**  $C_{22}H_{18}N_2$  [Same as the namesake monograph]

**Bile salts** See Microbial Limit Test for Crude Drugs <5.02>.

**2-(4-Biphenyl)propionic acid**  $C_{15}H_{14}O_2$  Light yellowish white powder.

*Melting point* <2.60>: 145 – 148°C

*Purity*—Dissolve 1 mg of 2-(4-biphenyl) propionic acid in a mixture of water and acetonitrile (11:9) to make 50 mL. Perform the test with 20  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions of the Related substances in the Purity (3) under Flurbiprofen. Determine each peak area of the solution in about twice as long as the retention time of the main peak by the automatic integration method, and calculate the amount of 2-(4-biphenyl)propionic acid by the area percentage method: it is not less than 98.0%.

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 0.5 g of 2-(4-biphenyl)propionic acid, previously dried in vacuum over silica gel for 4 hours, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 22.63 mg of  $C_{15}H_{14}O_2$

**2,2'-Bipyridyl**  $C_{10}H_8N_2$  [K 8486, Special class]

**Bis(cis-3,3,5-trimethylcyclohexyl) phthalate**  
 $C_6H_4[COOC_6H_8(CH_3)_3]_2$  White crystalline powder.  
*Melting point* <2.60>: 91 – 94°C

**Bisdemethoxycurcumin**  $C_{19}H_{16}O_4$  Yellow to orange crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. *Melting point*: 213 – 217°C.

*Identification*—Determine the absorption spectrum of a solution of bisdemethoxycurcumin in methanol (1 in 400,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 413 nm and 417 nm.

*Purity* Related substances—(1) Dissolve 4 mg of bisdemethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at *R<sub>f</sub>* value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of bisdemethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography

<2.01> under the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of bisdemethoxycurcumin obtained from the sample solution is not larger than the peak area of bisdemethoxycurcumin from the standard solution.

*Operating conditions*

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

*Detector*: A visible absorption photometer (wavelength: 422 nm).

*Time span of measurement*: About 4 times as long as the retention time of bisdemethoxycurcumin beginning after the solvent peak.

*System suitability*

*Test for required detectability*: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of bisdemethoxycurcumin obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of bisdemethoxycurcumin from 10  $\mu$ L of the standard solution.

*System performance and system repeatability*: Proceed as directed in the operating conditions in the Assay under Turmeric.

**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 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 16.22 mg of  $C_{21}H_{28}N_2O$

***N,N'*-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide**  $C_{16}H_{20}I_3N_3O_8$   
White crystalline powder.

*Identification* (1) Heat 0.1 g of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide over free flame: a purple colored gas evolves.

(2) Determine the infrared absorption spectrum of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390  $cm^{-1}$ , 3230  $cm^{-1}$ , 2882  $cm^{-1}$ , 1637  $cm^{-1}$ , 1540  $cm^{-1}$ , 1356  $cm^{-1}$  and 1053  $cm^{-1}$ .

*Purity*—Dissolve 0.10 g of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide obtained from the sample solution is not larger than 3 times of the

peak area of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetyl-amino-2,4,6-triiodoisophthalamide obtained from the standard solution.

**Operating conditions**

Proceed the operating conditions in the Purity (6) under Iopamidol.

**System suitability**

Proceed the system suitability in the Purity (6) under Iopamidol.

**Bismuth nitrate** See bismuth nitrate pentahydrate.

**Bismuth nitrate pentahydrate**  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  [K 8566, 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, and filter (solution A). Separately, dissolve 10 g of *L*-tartaric acid in 50 mL of water, add 5 mL of solution A, and preserve in a light-resistant, glass-stoppered bottle.

**Bismuth sodium trioxide**  $\text{NaBiO}_3$  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 <1.09> (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.

**Bismuth subnitrate-potassium iodide TS for spraying, dilute** Dissolve 10 g of *L*-tartaric acid in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

**Bismuth sulfite indicator** Prepared for microbial test.

**Bisoprolol fumarate for assay**  $(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  [Same as the monograph Bisoprolol Fumarate. However, when dried, it contains not less than 99.0% of bisoprolol fumarate  $[(\text{C}_{18}\text{H}_{31}\text{NO}_4)_2 \cdot \text{C}_4\text{H}_4\text{O}_4]$ . Also, when performing the Purity (2) under Bisoprolol Fumarate, the total area of the peaks other than bisoprolol is not larger than 1/5 times the peak area of bisoprolol from the standard solution].

Purify as follows if needed.

**Purification method**—Dissolve, with heating, 2 g of Bi-

soprolol Fumarate in 200 mL of ethyl acetate, add 0.5 g of activated carbon, shake well, and filter using a glass filter (G4). Place the filtrate in ice water for 2 hours while occasional shaking. Collect the crystals that precipitate out using a glass filter (G3). Dry the crystals obtained in vacuum at 80°C for 5 hours using phosphorus (V) oxide as a desiccant.

**Bis-(1-phenyl-3-methyl-5-pyrazolone)**  $\text{C}_{20}\text{H}_{18}\text{B}_4\text{O}_2$   
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.

*Nitrogen content* <1.08>: 15.5 – 16.5%

*Residue on ignition* <2.44>: not more than 0.1%.

**Bis(1,1-trifluoroacetoxy)iodobenzene**  $\text{C}_{10}\text{H}_5\text{F}_6\text{IO}_4$   
Prepared for amino acid analysis or biochemistry.

**Bis-trimethyl silyl acetamide**  $\text{CH}_3\text{CON}[\text{Si}(\text{CH}_3)_3]_2$   
Colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.414 – 1.418

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.825 – 0.835

*Boiling point* <2.57>: 71 – 73°C

**Bitter orange peel** [Same as the namesake monograph]

**Block buffer solution** Dissolve 4 g of blocking agent in 100 mL of water, and add 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4.

**Blocking agent** Powder whose main ingredient is bovine-derived lactoprotein. For immunological research purposes.

**Blood agar medium** Sterilize 950 mL of heart infusion agar medium under increased pressure. Allow the media to cool to about 50°C, add 50 mL of horse or sheep defibrinated blood, dispense in sterilized Petri dishes, and make them as plate media.

**1% blood suspension** Wash a defibrinated animal blood in isotonic solution, and make it into suspension to contain 1 vol%. Prepare before use.

**Blue tetrazolium**  $\text{C}_{40}\text{H}_{32}\text{Cl}_2\text{N}_8\text{O}_2$  3,3'-Dianisole-bis-[4,4'-(3,5-diphenyl) tetrazolium chloride] Light yellow crystals. Freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in water, and practically insoluble in acetone and in ether. Melting point: about 245°C (with decomposition).

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (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**  $\text{C}_5\text{H}_8\text{BN}$

*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 <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 1.549 mg of  $\text{C}_5\text{H}_8\text{BN}$

**Borate-hydrochloric acid buffer solution, pH 9.0** Dis-

solve 19.0 g of sodium borate in 900 mL of water, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Borax** See sodium tetraborate decahydrate.

**Boric acid**  $\text{H}_3\text{BO}_3$  [K 8863, Special class]

**Boric acid-magnesium chloride buffer solution, pH 9.0**

Dissolve 3.1 g of boric acid in 210 mL of dilute sodium hydroxide, and add 10 mL of magnesium chloride hexahydrate (1 in 50) and water to make 1000 mL. Adjust the pH to 9.0, if necessary.

**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 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 200 mL.

**Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6** To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 36.85 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

**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 for buffer solution add 43.90 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 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.

**Boric acid-sodium hydroxide buffer solution, pH 8.4**

Dissolve 24.736 g of boric acid in 0.1 mol/L sodium hydroxide VS to make exactly 1000 mL.

**Boron trifluoride**  $\text{BF}_3$  Colorless gas, having an irritating odor.

*Melting point* <2.60>:  $-127.1^\circ\text{C}$

*Boiling point* <2.57>:  $-100.3^\circ\text{C}$

**Boron trifluoride-methanol TS** A solution containing 14 w/v% of boron trifluoride ( $\text{BF}_3$ : 67.81) in methanol.

**Bovine activated blood coagulation factor X** A protein obtained from bovine plasma. It has an activity to decompose prothrombin specifically and limitedly and produce thrombin. It does not contain thrombin and plasmin. It contains not less than 500 Units per mg protein. One unit indicates an amount of the factor X which hydrolyzes  $1\ \mu\text{mol}$  of *N*-benzoyl-L-isoleucyl-L-glutamyl( $\gamma$ -OR)-glycyl-L-arginyl-*p*-nitroanilide in 1 minute at  $25^\circ\text{C}$ .

**Bovine serum** Serum obtained from blood of bovine. Interleukin-2 dependent cell growth suppression substance is

removed by heat at  $56^\circ\text{C}$  for 30 min before use

**Bovine serum albumin** Obtained from cattle serum as Cohn's fifth fraction. Contains not less than 95% of albumin.

**Bovine serum albumin for assay** White or yellowish crystals or crystalline powder.

Take 50 mg of bovine serum albumin containing 99% or more albumin in glass ampoules and put them in the desiccator, whose humidity is adjusted to 31%RH at  $25^\circ\text{C}$  with calcium chloride-saturated solution, for 2 weeks, and then take out and seal them immediately.

*Protein content*: not less than 88%. Assay—Weigh accurately about 0.1 g of bovine serum albumin for assay, dissolve in water, and add water to make exactly 20 mL. Put exactly 3 mL of the solution in the Kjeldahl flask, and determine protein content following Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS  
= 0.8754 mg protein

*Storage*—Store at  $4^\circ\text{C}$  or lower.

**Bovine serum albumin for test of ulinastatin** White crystalline powder obtained from bovine serum by a purification method which does not denature albumin and other serum proteins. It contains not less than 99% of albumin.

**0.1% Bovine serum albumin-acetate buffer solution** Dissolve 0.1 g of bovine serum albumin in a solution of sodium acetate trihydrate (1 in 100) to make exactly 100 mL, and adjust to pH 4.0 with 1 mol/L hydrochloric acid TS.

**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.

**1 w/v% Bovine serum albumin-phosphate buffer-sodium chloride TS** Dissolve 1 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4.

**Bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2** Dissolve 10.75 g of disodium hydrogen phosphate dodecahydrate, 7.6 g of sodium chloride and 1.0 g of bovine serum albumin in water to make 1000 mL. Adjust to pH 7.2 with dilute sodium hydroxide TS or diluted phosphoric acid (1 in 10) before use.

**Bovine serum albumin TS for secretin** Dissolve 0.1 g of bovine serum albumin, 0.1 g of L-cysteine hydrochloride monohydrate, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

**Bovine serum albumin TS for Secretin RS** Dissolve 0.1 g of bovine serum albumin, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

**Bradykinin**  $\text{C}_{50}\text{H}_{73}\text{N}_{15}\text{O}_{11}$  A white powder. Freely soluble in water and in acetic acid (31), and practically insoluble in diethyl ether.

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ :  $-80 - -90^\circ$  (15 mg, water, 5 mL, 100 mm).

**Purity** Related substances—Dissolve 2.0 mg of bradykinin in 0.2 mL of water, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu\text{L}$  of the sample solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (31) (15:12:10:3) to a distance of about 10 cm, and dry the plate at 60°C. Spray evenly a solution of ninhydrin in 1-butanol (1 in 1000) on the plate, and heat at 60°C for 30 to 60 minutes: any spot other than the principal spot arisen from bradykinin does not appear.

**Brilliant green**  $\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_4\text{S}$  Fine, glistening, yellow crystals. It dissolves in water and in ethanol (95). The wavelength of absorption maximum: 623 nm.

**Bromine** Br [K 8529, Special class]

**Bromine-acetic acid TS** Dissolve 10 g of sodium acetate trihydrate in acetic acid (100) to make 100 mL, add 5 mL of bromine, and shake. Preserve in light-resistant containers, preferably in a cold place.

**Bromine-carbon tetrachloride TS** To 0.1 g of bromine add carbon tetrachloride to make 100 mL, and dilute a 2 mL portion of this solution with carbon tetrachloride to make 100 mL. Prepare before use.

**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-sodium hydroxide TS** To 100 mL of a solution of sodium hydroxide (3 in 100) add 0.2 mL of bromine. Prepare before use.

**Bromine TS** Prepare by saturating water with bromine as follows: Transfer 2 to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100 mL of cold water, insert the stopper, and shake. Preserve in light-resistant containers, preferably in a cold place.

**Bromocresol green**  $\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$  [K 8840, Special class]

**Bromocresol green-crystal violet TS** Dissolve 0.3 g of bromocresol green and 75 mg 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 portions 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.

**Bromocresol green-sodium hydroxide TS** Triturate 0.2 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 50 mg of bromocresol green in 100 mL of ethanol (95), and filter if necessary.

**Bromocresol purple**  $\text{C}_{21}\text{H}_{16}\text{Br}_2\text{O}_5\text{S}$  [K 8841, Special class]

**Bromocresol purple-dipotassium hydrogenphosphate-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 portions 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**  $\text{C}_{19}\text{H}_{10}\text{Br}_4\text{O}_5\text{S}$  [K 8844, Special class]

**Bromophenol blue-potassium biphthalate TS** Dissolve 0.1 g of bromophenol blue in potassium biphthalate buffer solution, pH 4.6, to make 100 mL.

**Bromophenol blue TS** Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol, and filter if necessary.

**0.05% Bromophenol blue TS** Dissolve 0.01 g of bromophenol blue in water to make 20 mL.

**Bromophenol blue TS, dilute** Dissolve 0.05 g of bromophenol blue in 100 mL of ethanol (99.5). Prepare before use.

**Bromophenol blue TS, pH 7.0** Mix 10 mL of bromophenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with dilute sodium hydroxide TS.

**N-Bromosuccinimide**  $\text{C}_4\text{H}_4\text{BrNO}_2$  [K 9553, Special class]

**N-Bromosuccinimide TS** Dissolve 1 g of *N*-bromosuccinimide in 1000 mL of water.

**Bromothymol blue**  $\text{C}_{27}\text{H}_{28}\text{Br}_2\text{O}_5\text{S}$  [K 8842, Special class]

**Bromothymol blue-sodium hydroxide TS** To 0.2 g of powdered bromothymol blue add 5 mL 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-sodium hydroxide-ethanol TS** Dissolve 50 mg of bromothymol blue in 4 mL of diluted 0.2 mol/L sodium hydroxide TS (1 in 10) and 20 mL of ethanol (99.5), and 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.

**Bromovalerylurea**  $\text{C}_6\text{H}_{11}\text{BrN}_2\text{O}_2$  [Same as the name-sake monograph]

**Brucine** See brucine *n*-hydrate.

**Brucine dihydrate** See brucine *n*-hydrate.

**Brucine *n*-hydrate**  $C_{23}H_{26}N_2O_4 \cdot nH_2O$  [K 8832, 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.

**Bucillamine**  $C_7H_{13}NO_3S_2$  [Same as the namesake monograph]

**Bucillamine for assay**  $C_7H_{13}NO_3S_2$  [Same as the monograph Bucillamine. However, when dried, it contains not less than 99.0% of bucillamine ( $C_7H_{13}NO_3S_2$ ). Furthermore, it conforms to the following test.]

**Purity** Related substances—Dissolve 60 mg of bucillamine for assay in 20 mL of a mixture of water and methanol (1:1) and use this solution as the sample solution. Pipet 1 mL of this solution, add the mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. When the test is performed according to the Purity (3) under Bucillamine, the total area of the peaks other than the bucillamine peak from the sample solution is not larger than the peak area of bucillamine from the standard solution.

**Bufalin for assay**  $C_{24}H_{34}O_4 \cdot xH_2O$  White, odorless, crystalline powder.

**Absorbance**  $\langle 2.24 \rangle$   $E_{1\text{cm}}^{1\%}$  (300 nm): 143 – 153 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

**Purity** Related substances—Dissolve 40 mg of bufalin for assay in 5 mL of chloroform and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography  $\langle 2.03 \rangle$ . Spot 5  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and chloroform (4:3:3) to a distance of about 14 cm, and air-dry. Spray evenly sulfuric acid, and heat at 100°C for 2 to 3 minutes: any spot other than the principal spot obtained from the sample solution is not larger and not more intense than the spot from the standard solution.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 10 mg of bufalin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography  $\langle 2.01 \rangle$  according to the following conditions. Measure the peak area by the automatic integration method, and calculate the amount of bufalin by the area percentage method.

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 300 nm).

**Column:** A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10  $\mu\text{m}$  in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of water and acetonitrile (1:1).

**Flow rate:** Adjust the flow rate so that the retention time of bufalin is about 6 minutes.

**Selection of column:** Dissolve 0.01 g each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Proceed with 20  $\mu\text{L}$  of this solution according to the above conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order and completely resolving these peaks.

**Detection sensitivity:** Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of bufalin obtained from 20  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of bufalin from 20  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About twice as long as the retention time of bufalin beginning after the solvent peak.

**Bufalin for component determination** See bufalin for assay.

**Buffer solution for celmoleukin** Combine 12.5 mL of 0.5 mol/L tris buffer solution, pH 6.8, 10 mL of sodium lauryl sulfate solution (1 $\rightarrow$ 10), 10 mL of glycerin, and 17.5 mL of water, shake, and then add and dissolve 5 mg of bromophenol blue.

**Storage**—Store in a cool place, shielded from light.

**Buformin hydrochloride for assay**  $C_6H_{15}N_5 \cdot \text{HCl}$  [Same as the monograph Buformin Hydrochloride. When dried, it contains not less than 99.5% of buformin hydrochloride ( $C_6H_{15}N_5 \cdot \text{HCl}$ ).]

***n*-Butanol** See 1-butanol.

***sec*-Butanol** See 2-butanol.

**1-Butanol**  $\text{CH}_3(\text{CH}_2)_2\text{CH}_2\text{OH}$  [K 8810, Special class]

**2-Butanol**  $\text{CH}_3\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$  [K 8812, Special class]

**2-Butanone**  $\text{CH}_3\text{COC}_2\text{H}_5$  [K 8900, Special class]

**Butenafine hydrochloride for assay**  $\text{C}_{23}\text{H}_{27}\text{N} \cdot \text{HCl}$  [Same as the monograph Butenafine Hydrochloride]

***N*-*t*-Butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester**  $\text{C}_{16}\text{H}_{21}\text{NO}_6$  White powder.

**Melting point**  $\langle 2.60 \rangle$ : 95–104°C

**Purity** Related substances—Dissolve 10 mg of *N*-*t*-butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography  $\langle 2.03 \rangle$ . Spot 10  $\mu\text{L}$  each of the sample solution and standard solution on three plates of silica gel with fluorescent indicator for thin-layer chromatography. Develop the first plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (25:25:1), the second plate with a mixture of benzene, 1,4-dioxane and acetic acid (100) (95:25:4), and the third plate with a mixture of chloroform, methanol and acetic acid (100) (45:4:1) to a distance of about 12 cm, and air-dry these plates. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard so-

lution in all plates.

**n-Butyl acetate**  $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$  [K 8377, Special class]

**t-Butyl alcohol**  $(\text{CH}_3)_3\text{COH}$  A crystalline solid, having a characteristic odor. A colorless liquid at above an ordinary temperature.

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.78; Boiling point: about 83°C; Melting point: about 25°C.

*Identification*—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3370  $\text{cm}^{-1}$ , 2970  $\text{cm}^{-1}$ , 1471  $\text{cm}^{-1}$ , 1202  $\text{cm}^{-1}$ , 1022  $\text{cm}^{-1}$ , 913  $\text{cm}^{-1}$  and 749  $\text{cm}^{-1}$ .

**n-Butylamine**  $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$  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* <2.56>  $d_{20}^{20}$ : 0.740 – 0.747

*Distilling range* <2.57>: 76.5 – 79°C, not less than 96 vol%.

**Butyl benzoate**  $\text{C}_6\text{H}_5\text{COOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$  A clear and colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.495 – 1.500

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.006 – 1.013

**n-Butylboronic acid**  $\text{C}_4\text{H}_9\text{BO}_2$  White flakes.

*Melting point* <2.60>: 90 – 92°C

**n-Butyl chloride** See 1-chlorobutane.

**n-Butyl formate**  $\text{HCOO}(\text{CH}_2)_3\text{CH}_3$  Clear and colorless liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.884 – 0.904

**tert-Butyl methyl ether**  $(\text{CH}_3)_3\text{COCH}_3$  Clear colorless liquid, having a specific odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.3689

*Specific gravity* <2.56>  $d_4^{20}$ : 0.7404

**Butyl parahydroxybenzoate**  $\text{HOC}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$  [Same as the namesake monograph]

**Butyrolactone**  $\text{C}_4\text{H}_6\text{O}_2$  Clear, colorless to practically colorless liquid.

*Specific gravity* <2.56>  $d_4^{25}$ : 1.128 – 1.135

*Boiling point* <2.57>: 198 – 208°C

**Cadmium acetate** See cadmium acetate dihydrate.

**Cadmium acetate dihydrate**  $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  White crystals or crystalline powder.

*Identification*—(1) Dissolve 0.2 g of cadmium acetate dihydrate in 20 mL of water, and use this as the 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 [H 2113, First class]

**Cadmium-ninhydrin TS** Dissolve 0.05 g of cadmium acetate dihydrate in 5 mL of water and 1 mL of acetic acid (100), add 2-butanone to make 50 mL, and dissolve 0.1 g of ninhydrin in this solution. Prepare before use.

**Cadralazine for assay**  $\text{C}_{12}\text{H}_{21}\text{N}_5\text{O}_3$  [Same as the monograph Cadralazine. When dried, it contains not less than 99.0% of cadralazine ( $\text{C}_{12}\text{H}_{21}\text{N}_5\text{O}_3$ ).]

**Caffeine** See caffeine hydrate.

**Caffeine hydrate**  $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2 \cdot \text{H}_2\text{O}$  [Same as the name-sake monograph]

**Caffeine, anhydrous**  $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$  [Same as the monograph Anhydrous Caffeine]

**Calcium acetate monohydrate**  $(\text{CH}_3\text{COO})_2\text{Ca} \cdot \text{H}_2\text{O}$  [K 8364, Special class]

**Calcium carbonate**  $\text{CaCO}_3$  [K 8617, Special class]

**Calcium carbonate for assay**  $\text{CaCO}_3$  [Same as the monograph Precipitated Calcium Carbonate. When dried, it contains not less than 99.0% of calcium carbonate ( $\text{CaCO}_3$ ).]

**Calcium chloride** See calcium chloride dihydrate.

**Calcium chloride dihydrate**  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  [K 8122, Special class]

**Calcium chloride for drying**  $\text{CaCl}_2$  [K 8124, For drying]

**Calcium chloride for Karl Fischer method**  $\text{CaCl}_2$  [K 8125, 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 gluconate for thin-layer chromatography** See calcium gluconate hydrate for thin-layer chromatography.

**Calcium gluconate hydrate for thin-layer chromatography** [Same as the monograph Calcium Gluconate Hydrate. When the test is performed as directed in the Identification (1) under Calcium Gluconate Hydrate, no spot other than the principal spot appears.]

**Calcium hydroxide**  $\text{Ca}(\text{OH})_2$  [K 8575, Special class]

**Calcium hydroxide for pH determination** Calcium hydroxide prepared for pH determination.

**Calcium hydroxide pH standard solution** See pH Determination <2.54>.

**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**  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  [K 8549, Special class]

**Calcium oxide**  $\text{CaO}$  [K 8410, Special class]

**Calcium paraaminosalicylate hydrate for assay**  $\text{C}_7\text{H}_5\text{CaNO}_3 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  [Same as the monograph Calcium Paraaminosalicylate Hydrate. It contains not less than 99.0% of calcium paraaminosalicylate ( $\text{C}_7\text{H}_5\text{CaNO}_3$ ), calculated on the anhydrous basis.]

**Camphor**  $\text{C}_{10}\text{H}_{16}\text{O}$  [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** <2.41>: not more than 2.0% (1 g, 105°C, 5 hours).

**Content**: not less than 99.0%, calculated on the dried basis. **Assay**—Weigh accurately about 4 g of *d*-camphorsulfonic acid, dissolve in 50 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS  
= 232.3 mg of  $C_{10}H_{16}O_4S$

**Candesartan cilexetil for assay**  $C_{33}H_{34}N_6O_6$  [Same as the monograph Candesartan Cilexetil. It contains not less than 99.5% of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ), calculated on the anhydrous basis, and when performed the test as directed in the Purity (2) under Candesartan Cilexetil, the total area of the peaks other than candesartan cilexetil obtained from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution.]

**Caprylic acid**  $CH_3(CH_2)_6COOH$  A clear and colorless oily liquid, having a slight unpleasant odor. Freely soluble in ethanol (95) and in chloroform, and very slightly soluble in water.

**Refractive index** <2.45>  $n_D^{20}$ : 1.426 – 1.430

**Specific gravity** <2.56>  $d_4^{20}$ : 0.908 – 0.912

**Distilling range** <2.57>: 238 – 242°C, not less than 95 vol%.

**Capsaicin for assay** See (*E*)-capsaicin for assay.

**(*E*)-Capsaicin for assay**  $C_{18}H_{27}NO_3$  Use (*E*)-capsaicin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (281 nm): 97 – 105 (10 mg, methanol, 200 mL). Use the sample dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours for the test.

**Purity** Related substances—Dissolve 10 mg of capsaicin for assay in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of the peaks other than capsaicin from the sample solution is not larger than the peak area of capsaicin from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed the operating conditions in the Assay under Capsicum.

Time span of measurement: About 3 times as long as the retention time of capsaicin beginning after the solvent peak. System suitability

System performance, and system repeatability: Proceed

the system suitability in the Assay under Capsicum.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of capsaicin from 20  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that of capsaicin from 20  $\mu\text{L}$  of the standard solution.

**(*E*)-Capsaicin for component determination** See (*E*)-capsaicin for assay.

**Capsaicin for thin-layer chromatography** See (*E*)-capsaicin for thin-layer chromatography.

**(*E*)-Capsaicin for thin-layer chromatography**  $C_{18}H_{27}NO_3$  White crystals, having a strong irritative odor. Very soluble in methanol, freely soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Melting point** <2.60>: 65 – 70°C

**Purity** Related substances—Dissolve 20 mg of (*E*)-capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Capsicum: any spot other than the principal spot (*R<sub>f</sub>* value is about 0.5) from the sample solution is not more intense than the spot from the standard solution.

**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 <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 23.62 mg of  $C_{10}H_{12}N_4O_3$

**Carbazochrome sodium sulfonate for component determination** See carbazochrome sodium sulfonate trihydrate.

**Carbazochrome sodium sulfonate trihydrate**

$C_{10}H_{11}N_4NaO_5S \cdot 3H_2O$  [Same as the monograph Carbazochrome Sodium Sulfonate Hydrate. It contains not less than 99.0% of carbazochrome sodium sulfonate ( $C_{10}H_{11}N_4NaO_5S$ ), calculated on the anhydrous basis, and meets the following additional requirement.]

**Water** <2.48>: 14.0 – 15.0%

**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** <2.60>: 243 – 245°C

**Purity** Clarity and color of solution—To 0.5 g of carbazole add 20 mL of ethanol (99.5), and dissolve by warming: the solution is clear.

Residue on ignition: Not more than 0.1% (1 g).

**Carbazole TS** Dissolve 0.125 g of carbazole in ethanol (99.5) to make 100 mL.

**0.1 mol/L Carbonate buffer solution, pH 9.6** Dissolve

3.18 g of anhydrous sodium carbonate and 5.88 g of sodium hydrogen carbonate in water to make 1000 mL.

**Carbon dioxide**  $\text{CO}_2$  [Same as the namesake monograph]

**Carbon disulfide**  $\text{CS}_2$  [K 8732, Special class]  
Preserve in tightly stoppered containers in a dark, cold place, remote from fire.

**Carbonic anhydrase** White powder. Derived from bovine RBC. Molecular weight about 29,000.

**Carbon monoxide**  $\text{CO}$  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**  $\text{CCl}_4$  [K 8459, Special class]

**Carvedilol for assay**  $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_4$  [Same as the monograph Carvedilol]

**Casein, milk** 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 <2.25>: it exhibits absorption at the wave numbers of about  $1650\text{ cm}^{-1}$ ,  $1540\text{ cm}^{-1}$  and  $1250\text{ cm}^{-1}$ .

**Casein (milk origin)** See casein, milk.

**Casein peptone** See peptone, casein.

**Castor oil** [Same as the namesake monograph]

**Catechol**  $\text{C}_6\text{H}_4(\text{OH})_2$  White crystals.  
*Melting point* <2.60>:  $104 - 107^\circ\text{C}$ .

Preserve in a light-resistant tight container.

**Cefadroxil**  $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$  [Same as the namesake monograph]

**Cefatrizine propylene glycolate**  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2 \cdot \text{C}_3\text{H}_8\text{O}_2$   
[Same as the namesake monograph]

**Cefcapene pivoxil hydrochloride** See cefcapene pivoxil hydrochloride hydrate.

**Cefcapene pivoxil hydrochloride hydrate**  
 $\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_8\text{S}_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$  [Same as the namesake monograph]

**Cefdinir lactam ring-cleavage lactones**  $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}_6\text{S}_2$  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 Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $1743\text{ cm}^{-1}$ ,  $1330\text{ cm}^{-1}$ ,  $1163\text{ cm}^{-1}$  and  $1047\text{ cm}^{-1}$ .

*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  $\mu\text{L}$  of the sample solution as directed in the operating conditions of the Purity (2) Related substances under Cefdinir, and calculate the areas of each peak by the automatic integration method. Determine the percent of the total peak area of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

**Cell suspension solution for teceleukin** Centrifuge for 5

minutes at 1000 r.p.m culture medium of NK-7 cells that have been cultured statically for 2 to 4 hours. Remove the supernatant by aspiration, and add culture medium for assay of teceleukin to a cell concentration of 2 to  $4 \times 10^5$  cells/mL.

#### Celmoleukin for liquid chromatography

$\text{C}_{693}\text{H}_{1118}\text{N}_{178}\text{O}_{203}\text{S}_7$  [Same as the monograph Celmoleukin (Genetical Recombination). However, contains 0.5 to 1.5 mg of protein per mL, polymers amount for 0.5% or less, and conforms to the following test].

*Identification* (1) When the amino acid sequence is investigated using the Edman technique and liquid chromatography, the amino acids are detected in the following sequence: alanine, proline, threonine, serine, serine, serine, threonine, lysine, lysine, threonine, glutamine, leucine, glutamine, leucine, and glutamic acid. Also, based on the results of the protein content determination test, place an amount of celmoleukin equivalent to about 0.3 mg in a hydrolysis tube, evaporate to dryness under vacuum, and then add 100  $\mu\text{L}$  of hydrazine anhydride for amino acid sequence analysis. Reduce the internal pressure of the hydrolysis tube by heating for 6 hours at about  $100^\circ\text{C}$ . After evaporating to dryness under vacuum, add 250  $\mu\text{L}$  of water to dissolve the residue. To this add 200  $\mu\text{L}$  of benzaldehyde, shake occasionally, leave for one hour, centrifuge, and remove the aqueous layer. Add 250  $\mu\text{L}$  of water to the benzaldehyde layer, shake, centrifuge, combine the aqueous layers, and evaporate to dryness under vacuum. Threonine is detected when amino acid analysis is conducted using the postcolumn technique with ninhydrin on a solution of the residue dissolved by adding 100  $\mu\text{L}$  of 0.02 mol/L hydrochloric acid TS.

(2) Add 1 mL of protein digestive enzyme solution to 1 mL of celmoleukin, shake, and leave for 18 to 24 hours at  $37^\circ\text{C}$ . Pipet 1 mL of this solution and add 25  $\mu\text{L}$  of trifluoroacetic acid (1 in 10). To another 1 mL, add 10  $\mu\text{L}$  of 2-mercaptoethanol, leave for 30 minutes at  $37^\circ\text{C}$ , and then add 25  $\mu\text{L}$  of trifluoroacetic acid (1 in 10). Perform Liquid Chromatography <2.01> on these two solutions separately under the conditions outlined in Celmoleukin (Genetical Recombination), Identification (4). Repeatedly pipet the celmoleukin derived peak fraction that elutes and when the test is performed according to Celmoleukin (Genetical Recombination), Identification (2), except for the lysines in positions 9 and 49 from the amino terminal amino acid, a peptide estimated from the complete primary structure is detected.

**Cephaeline hydrobromate**  $\text{C}_{28}\text{H}_{38}\text{N}_2\text{O}_4 \cdot 2\text{HBr} \cdot x\text{H}_2\text{O}$  A white or light-yellow crystalline powder.

*Purity*—Dissolve 10 mg in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Assay under Ipecac: when measure the peak areas 2 times as long as the retention time of emetine, the total area of the peaks other than cephaeline is not larger than the peak area of cephaeline from the standard solution.

**Ceric ammonium sulfate** See cerium (IV) tetraammonium sulfate dihydrate.

**Ceric ammonium sulfate-phosphoric acid TS** See cerium

(IV) tetraammonium sulfate-phosphoric acid TS.

**Ceric ammonium sulfate TS** See cerium (IV) tetraammonium sulfate TS.

**Cerium (III) nitrate hexahydrate**  $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  A colorless or light yellow, crystalline powder. It dissolves in water.

*Purity* (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: 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 peroxodisulfate, dissolve, and boil it for 15 minutes. After cooling, add 2 drops of 1,10-phenanthroline TS, and titrate <2.50> with 0.1 mol/L ferrous ammonium sulfate VS until the pale blue color of the solution changes to red.

Each mL of 0.1 mol/L ferrous ammonium sulfate VS  
= 43.42 mg of  $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$

**Cerium (III) nitrate TS** Dissolve 0.44 g of cerium (III) nitrate hexahydrate in water to make 1000 mL.

**Cerium (IV) diammonium nitrate**  $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$   
[K 8556, Special class]

**Cerium (IV) diammonium nitrate TS** Dissolve 6.25 g of cerium (IV) diammonium nitrate in 160 mL of diluted dilute nitric acid (9 in 50). Use within 3 days.

**Cerium (IV) sulfate tetrahydrate**  $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$   
[K 8976, Special class]

**Cerium (IV) tetraammonium sulfate dihydrate**  
 $\text{Ce}(\text{SO}_4)_2 \cdot 2(\text{NH}_4)_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$  [K 8977, Special class]

**Cerium (IV) tetraammonium sulfate-phosphoric acid TS** Dissolve 0.1 g of cerium (IV) tetraammonium sulfate in diluted phosphoric acid (4 in 5) to make 100 mL.

**Cerium (IV) tetraammonium sulfate TS** Dissolve 6.8 g of cerium (IV) tetraammonium sulfate in diluted sulfuric acid (3 in 100) to make 100 mL.

**Cerous nitrate** See cerium (III) nitrate hexahydrate.

**Cerous nitrate TS** See cerium (III) nitrate TS.

**Cesium chloride**  $\text{CsCl}$  White crystals or crystalline powder. Very soluble in water, and freely soluble in ethanol (99.5).

*Loss on drying* <2.41>: Not more than 1.0% (1 g, 110°C, 2 hours).

*Content*: not less than 99.0%. *Assay*—Weigh accurately about 0.5 g, previously dried, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS  
= 16.84 mg of  $\text{CsCl}$

**Cesium chloride TS** To 25.34 g of cesium chloride add water to make 1000 mL.

**Cetanol** [Same as the namesake monograph]

**Cetirizine hydrochloride for assay**  $\text{C}_{21}\text{H}_{25}\text{ClN}_2\text{O}_3 \cdot 2\text{HCl}$

[Same as the monograph Cetirizine Hydrochloride. When dried, it contains not less than 99.5% of cetirizine hydrochloride ( $\text{C}_{21}\text{H}_{25}\text{ClN}_2\text{O}_3 \cdot 2\text{HCl}$ ).]

**Cetrimide**  $\text{C}_{17}\text{H}_{38}\text{BrN}$  White to pale yellowish white powder, having a 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 10-mL portions 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 <2.50> 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.

Each mL of 0.05 mol/L potassium iodate VS  
= 33.64 mg of  $\text{C}_{17}\text{H}_{38}\text{BrN}$

#### **Chenodeoxycholic acid for thin-layer chromatography**

$\text{C}_{24}\text{H}_{40}\text{O}_4$  White crystals or crystalline powder. Very soluble in methanol and in acetic acid (100), freely soluble in ethanol (95), soluble in acetone, sparingly soluble in ethyl acetate, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 119°C (recrystallize from ethyl acetate).

*Purity* Related substances—Dissolve 25 mg 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  $\mu\text{L}$  of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot at the *R<sub>f</sub>* 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 <2.50> with 0.1 mol/L sodium hydroxide VS. Near the end point add 100 mL of freshly boiled and cooled water, and titrate again.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 39.26 mg of  $\text{C}_{24}\text{H}_{40}\text{O}_4$

#### **Chikusetsusaponin IV for thin-layer chromatography**

$\text{C}_{47}\text{H}_{74}\text{O}_{18} \cdot n\text{H}_2\text{O}$  White crystalline powder. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether. Melting point: about 215°C (with decomposition).

*Purity* Related substances—Dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and perform the test with 5  $\mu\text{L}$  of this solution as directed in the Identification under Panax Rhizome: any

spot other than the principal spot at the *Rf* value of about 0.4 does not appear.

**Chlomotropic acid** See disodium chlomotropate dihydrate.

**Chlomotropic acid TS** Dissolve 0.05 g of disodium chlomotropate 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.

**Chlomotropic acid TS, concentrated** Suspend 0.5 g of disodium chlomotropate dihydrate in 50 mL of sulfuric acid, centrifuge, and use the supernatant liquid. Prepare before use.

**Chloral hydrate**  $\text{CCl}_3\text{CH}(\text{OH})_2$  [Same as the namesake monograph]

**Chloral hydrate TS** Dissolve 5 g of chloral hydrate in 3 mL of water.

**Chloramine** See sodium toluenesulfonchloramide trihydrate.

**Chloramine TS** See sodium toluenesulfonchloramide TS.

**Chloramphenicol**  $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$  [Same as the monograph Chloramphenicol]

**Chlorauric acid** See hydrogen tetrachloroaurate (III) tetrahydrate.

**Chlorauric acid TS** See hydrogen tetrachloroaurate (III) tetrahydrate TS.

**Chlordiazepoxide**  $\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$  [Same as the namesake monograph]

**Chlordiazepoxide for assay**  $\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$  [Same as the monograph Chlordiazepoxide. When dried, it contains not less than 99.0% of  $\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$ ].

**Chlorhexidine hydrochloride**  $\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}\cdot 2\text{HCl}$  [Same as the namesake monograph]

**Chlorinated lime** [Same as the namesake monograph]

**Chlorinated lime TS** Triturate 1 g of chlorinated lime with 9 mL of water, and filter. Prepare before use.

**Chlorine**  $\text{Cl}_2$  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.

**Chloroacetic acid**  $\text{C}_2\text{H}_3\text{ClO}_2$  [K 8899, Special class]

***p*-Chloroaniline** See 4-chloroaniline

**4-Chloroaniline**  $\text{H}_2\text{NC}_6\text{H}_4\text{Cl}$  White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in hot water.

*Melting point* <2.60>: 70 – 72°C

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**4-Chlorobenzenediazonium TS** Dissolve 0.5 g of 4-chloroaniline in 1.5 mL of hydrochloric acid, and add water to

make 100 mL. To 10 mL of this solution add 10 mL of sodium nitrite TS and 5 mL of acetone. Prepare before use.

***p*-Chlorobenzene sulfonamide** See 4-chlorobenzene sulfonamide.

**4-Chlorobenzene sulfonamide**  $\text{ClC}_6\text{H}_4\text{SO}_2\text{NH}_2$  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 300 mL, and perform the test with 5  $\mu\text{L}$  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**  $\text{ClC}_6\text{H}_4\text{COOH}$  White crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically insoluble in water.

*Melting point* <2.60>: 238 – 242°C

*Content*: not less than 99.0%. Assay—Weigh accurately about 0.3 g of 4-chlorobenzoic acid, dissolve in 30 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 15.66 mg of  $\text{C}_7\text{H}_5\text{ClO}_2$

**1-Chlorobutane**  $\text{CH}_3(\text{CH}_2)_3\text{Cl}$  Clear and colorless liquid, miscible with ethanol (95) and with diethyl ether, practically insoluble in water.

*Refractive index* <2.45>  $n_D^{20}$ : 1.401 – 1.045

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.884 – 0.890

*Boiling point* <2.57>: about 78°C

**Chlorobutanol**  $\text{C}_4\text{H}_7\text{Cl}_3\text{O}$  [Same as the namesake monograph]

**1-Chloro-2,4-dinitrobenzene**  $\text{C}_6\text{H}_3(\text{NO}_2)_2\text{Cl}$  Light yellow crystals or crystalline powder.

*Melting point* <2.60>: 50 – 54°C.

Preserve in a light-resistant tight container.

**3'-Chloro-3'-deoxythymidine for liquid chromatography**

$\text{C}_{10}\text{H}_{13}\text{N}_2\text{O}_4\text{Cl}$  Occurs as a white powder.

*Purity*—Dissolve 10 mg of 3'-chloro-3'-deoxythymidine for liquid chromatography in the mobile phase to make 100 mL. Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Purity (3) under Zidovudine: a peak is not observed at the retention time for zidovudine.

**(2-Chloroethyl) diethylamine hydrochloride**

$\text{C}_6\text{H}_{14}\text{ClN}\cdot\text{HCl}$  White powder.

*Content*: not less than 95.0%. Assay—Weigh accurately about 0.2 g of (2-chloroethyl)diethylamine hydrochloride, previously dried at 45°C for 3 hours under reduced pressure, and dissolve in 15 mL of acetic acid (100). To this solution add 10 mL of a mixture of acetic acid (100) and mercury (II) acetate TS for nonaqueous titration (5:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 17.21 mg of  $\text{C}_6\text{H}_{14}\text{ClN}\cdot\text{HCl}$

**Chloroform**  $\text{CHCl}_3$  [K 8322, 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 portions 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.

**Chloroform for Karl Fischer method** See Water Determination <2.48>.

**Chlorogenic acid for thin-layer chromatography** See (*E*)-chlorogenic acid for thin-layer chromatography.

**(*E*)-Chlorogenic acid for thin-layer chromatography**  $\text{C}_{16}\text{H}_{18}\text{O}_9 \cdot x\text{H}_2\text{O}$  A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

**Purity** Related substances—Dissolve 1.0 mg of (*E*)-chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot at around *R<sub>f</sub>* 0.5 appears.

***p*-Chlorophenol** See 4-Chlorophenol.

**4-Chlorophenol**  $\text{ClC}_6\text{H}_4\text{OH}$  Colorless or pale red crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol (95), in chloroform, in diethyl ether and in 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 100 mL. Measure exactly 25 mL of this solution into an iodine flask, add exactly 20 mL of 0.05 mol/L bromine VS and then 5 mL of hydrochloric acid, stopper immediately, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5 mL of a solution of potassium iodide (1 in 5), stopper immediately, shake well, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS  
= 3.214 mg of  $\text{C}_6\text{H}_5\text{ClO}$

Preserve in tight, light-resistant containers.

**(2-Chlorophenyl)-diphenylmethanol for thin-layer chromatography**  $\text{C}_{19}\text{H}_{15}\text{ClO}$  To 5 g of clotrimazole add 300 mL of 0.2 mol/L hydrochloric acid TS, boil for 30 minutes, cool, and extract with 100 mL of diethyl ether. Wash the diethyl ether extract with two 10 mL portions of 0.2 mol/L hydrochloric acid TS, then with two 10-mL portions of water. Shake the diethyl ether extract with 5 g of anhydrous sodium sulfate, and filter. Evaporate the diethyl ether of the filtrate, dissolve the residue in 200 mL of methanol by warming, and filter. Warm the filtrate, and add gradually 100 mL of water by stirring. Cool in an ice bath, filter the separated crystals, and dry in a desiccator (phosphorus (V) oxide) for

24 hours. A white crystalline powder. Very soluble in dichloromethane, freely soluble in diethyl ether, soluble in methanol, and practically insoluble in water.

**Melting point** <2.60>: 92 – 95°C

**Purity** Related substances—Dissolve 10 mg of (2-chlorophenyl)-diphenylmethanol in dichloromethane to make exactly 20 mL, and perform the test with 10  $\mu\text{L}$  of this solution as directed in the Purity (7) under Clotrimazole: any spot other than the principal spot does not appear.

**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.

**Chlorphenesin carbamate for assay**  $\text{C}_{10}\text{H}_{12}\text{ClNO}_4$  [Same as the monograph Chlorphenesin Carbamate. When dried, it contains not less than 99.0% of chlorphenesin carbamate ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_4$ ).]

**Chlorpheniramine maleate**  $\text{C}_{16}\text{H}_{19}\text{ClN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$  [Same as the namesake monograph]

**Chlorpromazine hydrochloride for assay**  $\text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S} \cdot \text{HCl}$  [Same as the monograph Chlorpromazine Hydrochloride]

**Chlorpropamide for assay**  $\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$  [Same as the monograph Chlorpropamide. When dried, it contains not less than 99.0% of chlorpropamide ( $\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$ ).]

**Cholesterol**  $\text{C}_{27}\text{H}_{45}\text{OH}$  [Same as the namesake monograph]

**Cholesterol benzoate**  $\text{C}_{34}\text{H}_{50}\text{O}_2$  White crystalline powder.

**Melting point** <2.60>: 145 – 152°C

**Cholic acid for thin-layer chromatography**  $\text{C}_{24}\text{H}_{40}\text{O}_5$  White, crystals or crystalline powder. Soluble in acetic acid (100), sparingly soluble in acetone and in ethanol (95), and very slightly soluble in water. Melting point: about 198°C

**Purity** Related substances—Dissolve 25 mg in acetone to make exactly 250 mL. Proceed with 10  $\mu\text{L}$  of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot, having *R<sub>f</sub>* about 0.1, does not appear.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.5 g, previously dried at 80°C for 4 hours (in vacuum, phosphorous (V) oxide), 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 until immediately before the end-point has been reached. Then add 100 mL of freshly boiled and cooled water, and continue the titration <2.50>. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 40.86 mg of  $\text{C}_{24}\text{H}_{40}\text{O}_5$

**Choline chloride**  $[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}]\text{Cl}$  White crystalline powder.

**Melting point** <2.60>: 303 – 305°C (decomposition).

**Water** <2.48>: less than 0.1%.

**Chromic acid-sulfuric acid TS** Saturate chromium (VI) trioxide in sulfuric acid.

**Chromium trioxide** See chromium (VI) trioxide.

**Chromium trioxide TS** See chromium (VI) trioxide TS.

**Chromium (VI) trioxide**  $\text{CrO}_3$  A dark red-purple thin needle-shaped or inner prism-like crystals, or light masses.

**Identification**—To 5 mL of a solution (1 in 50) add 0.2 mL of lead (II) acetate TS: yellow precipitates appear which does not dissolve on the addition of acetic acid.

**Chromium (VI) trioxide TS** Dissolve 3 g of chromium (VI) trioxide in water to make 100 mL.

**Chromogenic synthetic substrate** Equal amount mixture of *N*-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride and *N*-benzoyl-L-isoleucyl- $\gamma$ -methoxy glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride. White or pale yellow masses or powder. It is slightly soluble in water.

**Identification**—Perform the test with the solution (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorption maximum at about 316 nm is observed.

**Purity** Free 4-nitroaniline: not more than 0.5%.

**Loss on drying** <2.41>: not more than 5% (0.2 g, reduced pressure (0.3 kPa), calcium chloride, between 30 and 40°C, 18 hours).

**Content**: not less than 95% and not more than 105% of the label.

**Chromophore TS for teceleukin** Mix 0.1 mL of diluted hydrogen peroxide (30) (1 in 20) with 10 mL of 0.2 mol/L citric acid buffer, pH 3.8, containing 0.2 mmol/L 3,3',5,5'-tetramethylbenzidine dihydrochloride dehydrate, and use immediately.

**Cibenzoline succinate for assay**  $\text{C}_{18}\text{H}_{18}\text{N}_2\cdot\text{C}_4\text{H}_6\text{O}_4$   
[Same as the monograph Cibenzoline Succinate. When dried, it contains not less than 99.0% of cibenzoline succinate ( $\text{C}_{18}\text{H}_{18}\text{N}_2\cdot\text{C}_4\text{H}_6\text{O}_4$ ) and meets the following requirement.]

**Purity** Related substances—Dissolve 0.10 g of cibenzoline succinate for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. To exactly 1 mL of this solution add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution. On standing the plate for 30 minutes in the tank saturated with iodine vapor, the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

**Cilastatin ammonium for assay**  $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_5\text{S}$ : 375.48

A white crystalline powder.

**Water** <2.48>: not more than 0.5% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** <2.44>: not more than 0.5% (1 g).

**Purity** Related substances—Dissolve 40 mg of the substance to be examined in 25 mL of water, and use this as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the each peak area by the automatic integration method. Separately, perform the test with 20  $\mu\text{L}$  of water in the same manner to correct any variance of the peak area caused the variation of the baseline: the total area of the peaks other than cilastatin is not larger than 1/6 times the peak area of cilastatin from the standard solution.

**Operating conditions**

**Detector**: An ultraviolet absorption photometer (wavelength: 210 nm).

**Column**: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu\text{m}$  in particle diameter).

**Column temperature**: A constant temperature of about 50°C.

**Mobile phase A**: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

**Mobile phase B**: Diluted phosphoric acid (1 in 1000).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	15 → 100	85 → 0
30 – 40	100	100

**Flow rate**: 2.0 mL per minute.

**Time span of measurement**: 40 minutes.

**System suitability**

**Test for required detectability**: To exactly 1 mL of the standard solution add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20  $\mu\text{L}$  of this solution is equivalent to 2.3 to 4.5% of that with 20  $\mu\text{L}$  of the standard solution.

**System performance**: When the procedure is run with 20  $\mu\text{L}$  of the standard solution under the above operating conditions: the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

**System repeatability**: When the test is repeated 3 times with 20  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 3.0%.

**Residual solvent**—Weigh accurately about 1 g, dissolve in water to make exactly 100 mL, and use this as the sample solution. Separately, weigh accurately about 0.10 g of ethanol (99.5), add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution as directed under

Gas Chromatography <2.02> according to the following conditions. Determine the peak areas,  $A_T$  and  $A_S$ , of ethanol by the automatic integration method, and calculate the amount of ethanol ( $C_2H_5OH$ ): not more than 0.5%.

$$\begin{aligned} & \text{Amount (\% of ethanol } (C_2H_5OH)) \\ &= \frac{M_S}{M_T} \times \frac{A_T}{A_S} \times 100 \end{aligned}$$

$M_S$ : Amount (mg) of ethanol (99.5)

$M_T$ : Amount (mg) of the sample

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.5 mm in inside diameter and 30 m in length, coated the inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 5  $\mu\text{m}$ .

Column temperature: Inject the sample at a constant temperature of about 50°C, keep on for 150 seconds, then raise to 70°C at the rate of 8°C per minute, and keep this for 30 seconds.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of ethanol is about 1 minute.

Sprit ratio: 5:1

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL, and designate this the solution for system suitability test. To exactly 1 mL of the solution for system suitability test add water to make exactly 10 mL. Confirm that the peak area of ethanol obtained with 1  $\mu\text{L}$  of this solution is equivalent to 7 to 13% of that with 1  $\mu\text{L}$  of the solution for system suitability test.

System performance: When the procedure is run with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethanol are not less than 1500 and not more than 3.0, respectively.

System repeatability: When determine the peak area of methanol by repeating 6 times with 1  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0%.

**Content:** not less than 99.0% of cilastatin ammonium ( $C_{16}H_{29}N_3O_5S$ ), calculated on the anhydrous basis and corrected on the amount of ethanol. **Assay**—Weigh accurately about 0.5 g, dissolve in 30 mL of methanol, and add 5 mL of water. Adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the second equivalence point (potentiometric titration).

$$\begin{aligned} & \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ &= 37.55 \text{ mg of } C_{16}H_{29}N_3O_5S \end{aligned}$$

**Cilazapril** See cilazapril hydrate.

**Cilazapril hydrate**  $C_{22}H_{31}N_3O_5 \cdot H_2O$  [Same as the namesake monograph]

**Cilazapril for assay** See cilazapril hydrate for assay.

**Cilazapril hydrate for assay**  $C_{22}H_{31}N_3O_5 \cdot H_2O$  [Same as the monograph Cilazapril Hydrate. It contains not less than 99.0% of cilazapril ( $C_{22}H_{31}N_3O_5$ ), calculated on the anhy-

drous basis.]

**Cinchonidine**  $C_{19}H_{22}N_2O$  White crystals or crystalline powder. Soluble in ethanol (95), in methanol and in chloroform, sparingly soluble in diethyl ether, and practically insoluble 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 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 14.72 \text{ mg of } C_{19}H_{22}N_2O \end{aligned}$$

**Cinchonine**  $C_{19}H_{22}N_2O$  White crystals or powder.

**Identification**—Dissolve 1 g in 20 mL of diluted hydrochloric acid (1 in 4), and add 2 mL of potassium hexacyanoferrate (II) TS: yellow precipitates appear, which are dissolved by heating, and crystals are formed after allowing to cool.

**Purity** Cinchonidine and quinine—To 1 g add 30 mL of water, add diluted hydrochloric acid (2 in 3) dropwise until the substance to be tested dissolves, and neutralize with ammonia TS. To this solution add 10 mL of a solution of sodium tartrate dihydrate (1 in 2), boil, and allow to stand for 1 hour: no precipitates appear.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.3 g, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 14.72 \text{ mg of } C_{19}H_{22}N_2O \end{aligned}$$

**Cineol for assay**  $C_{10}H_{18}O$  Clear and colorless liquid, having a characteristic aroma.

**Refractive index** <2.45>  $n_D^{20}$ : 1.457 – 1.459

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.920 – 0.930

**Purity** (1) Related substances (i)—Dissolve 0.20 g of cineol for assay in 10 mL of hexane and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu\text{L}$  of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:1) to a distance of about 10 cm, and air-dry. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS, and heat at 105°C for 5 minutes: any spot other than the principal spot does not appear.

(2) Related substances (ii)—Dissolve 0.10 g of cineol for assay in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2  $\mu\text{L}$  of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cineol by the area percentage method: it is not less than 99.0%.

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 the sample solution and add hexane to make 100 mL. Adjust the detection sen-

sitivity so that the peak height of cineol obtained from 2  $\mu$ L 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 cineol beginning after the solvent peak.

**Cinnamaldehyde for thin-layer chromatography** See (*E*)-cinnamaldehyde for thin-layer chromatography.

**(*E*)-Cinnamaldehyde for thin-layer chromatography**

$C_9H_8O$  A colorless or light yellow liquid, having a characteristic aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (285 nm): 1679 – 1943 (5 mg, methanol, 2000 mL).

*Purity* Related substances—Dissolve 10 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 2 mL of methanol. Perform the test with 1  $\mu$ L of this solution as directed in the Identification (3) under Kakkonto Extract: no spot other than the principal spot (*Rf* value is about 0.4) appears.

**Cinnamic acid**  $C_9H_8O_2$  White crystalline powder, having a characteristic odor.

*Melting point* <2.60>: 132 – 135°C

**(*E*)-Cinnamic acid for assay** (*E*)-Cinnamic acid for thin-layer chromatography. It meets the following requirements.

*Purity* Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than (*E*)-cinnamic acid and the solvent is not larger than the peak area of (*E*)-cinnamic acid obtained with the standard solution.

*Operating conditions*

*Detector, column, column temperature, mobile phase, and flow rate:* Proceed as directed in the operating conditions in the Assay (1) under Ryokeijutsukanto Extract.

*Time span of measurement:* About 6 times as long as the retention time of (*E*)-cinnamic acid.

*System suitability*

*Test for required detectability:* To exactly measured 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of (*E*)-cinnamic acid obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

*System performance, and system repeatability:* Proceed as directed in the system suitability in the Assay (1) under Ryokeijutsukanto Extract.

**(*E*)-Cinnamic acid for thin-layer chromatography**

$C_9H_8O_2$  White crystals or crystalline powder, having a characteristic aromatic odor. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

*Melting point* <2.60>: 132 – 136°C

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (273 nm): 1307 – 1547 (5 mg dried with silica gel for 24 hours, methanol, 1000 mL).

*Purity* Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dis-

solve 10 mg in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (1) under Ryokeijutsukanto Extract: the spot other than the principal spot of around *Rf* 0.5 is not more intense than the spot obtained with the standard solution.

**(*E*)-Cinnamic acid for component determination** See (*E*)-cinnamic acid for assay.

**Cinobufagin for assay**  $C_{26}H_{34}O_6 \cdot xH_2O$  White crystalline odorless powder.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (295 nm): 125 – 127 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

*Purity* Related substances—Proceed with 40 mg of cinobufagin for assay as directed in the Purity under bufalin for component determination.

*Content:* not less than 98.0%. *Assay*—Weigh accurately about 10 mg of cinobufagin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cinobufagin by the area percentage method.

*Operating conditions*

*Detector:* Ultraviolet absorption photometer (wavelength: 295 nm).

*Column:* A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 to 10  $\mu$ m in particle diameter).

*Column temperature:* A constant temperature of about 40°C.

*Mobile phase:* A mixture of water and acetonitrile (1:1).

*Flow rate:* Adjust the flow rate so that the retention time of cinobufagin is about 7 minutes.

*Selection of column:* Dissolve 10 mg each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Proceed with 20  $\mu$ L of this solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

*Detection sensitivity:* Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of cinobufagin obtained from 20  $\mu$ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of cinobufagin from 20  $\mu$ L of the standard solution (1) is about 20% of the full scale.

*Time span of measurement:* About twice as long as the retention time of cinobufagin beginning after the solvent peak.

**Cinobufagin for component determination** See cinobufagin for assay.

**Cinoxacin for assay**  $C_{12}H_{10}N_2O_5$  [Same as the mono-

graph Cinoxacin. When dried, it contains not less than 99.0% of cinoxacin ( $C_{12}H_{10}N_2O_5$ ).]

**Cisplatin**  $Cl_2H_6N_2Pt$  [Same as the namesake monograph]

**Citric acid** See citric acid monohydrate.

**Citric acid-acetic acid TS** To 1 g of citric acid monohydrate add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve under shaking.

**Citric acid-acetic anhydride TS** To 1 g of citric acid monohydrate add 50 mL of acetic anhydride, and dissolve by heating. Prepare before use.

**Citric acid monohydrate**  $C_6H_8O_7 \cdot H_2O$  [K 8283, or same as the monograph Citric Acid Hydrate]

**Citric acid-phosphate-acetonitrile TS** Dissolve 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1).

**0.01 mol/L Citric acid TS** Dissolve 2.1 g of citric acid monohydrate in water to make 1000 mL.

**1 mol/L Citric acid TS for buffer solution** Dissolve 210.14 g of citric acid monohydrate in water to make 1000 mL.

**Clofibrate**  $C_{12}H_{15}ClO_3$  [Same as the namesake monograph]

**Clorazepate dipotassium for assay**  $C_{16}H_{10}ClKN_2O_3 \cdot KOH$  [Same as the monograph Clorazepate Dipotassium. When dried it contains not less than 99.0% of clorazepate dipotassium ( $C_{16}H_{10}ClKN_2O_3 \cdot KOH$ ).]

**Clotrimazole**  $C_{22}H_{17}ClN_2$  [Same as the namesake monograph]

**Cloxacolam**  $C_{17}H_{14}Cl_2N_2O_2$  [Same as the namesake monograph]

**Cobalt (II) chloride-ethanol TS** Dissolve 0.5 g of cobalt (II) chloride hexahydrate, previously dried at 105°C for 2 hours, in ethanol (99.5) to make 100 mL.

**Cobalt (II) chloride hexahydrate**  $CoCl_2 \cdot 6H_2O$  [K 8129, Special class]

**Cobalt (II) chloride TS** Dissolve 2 g of cobalt (II) chloride hexahydrate in 1 mL of hydrochloric acid and water to make 100 mL (0.08 mol/L).

**Cobalt (II) nitrate hexahydrate**  $Co(NO_3)_2 \cdot 6H_2O$  [K 8552, Special class]

**Cobaltous chloride** See cobalt (II) chloride hexahydrate.

**Cobaltous nitrate** See cobalt (II) nitrate hexahydrate.

**Codeine phosphate for assay** See codeine phosphate hydrate for assay.

**Codeine phosphate hydrate for assay**  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  [Same as the monograph Codeine Phosphate Hydrate. It contains not less than 99.0% of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4$ ), calculated on the anhydrous basis.]

**Collodion** Clear, colorless, viscous liquid, having a

diethyl ether-like odor.

*pH* <2.54>: 5.0–8.0

Stir 5 g of collodion while warming, add 10 mL of water gradually, and dry at 110°C after evaporating to dryness: mass of the residue is 0.250–0.275 g.

**Concentrated chromotropic acid TS** See chromotropic acid, concentrated.

**Concentrated diazobenzenesulfonic acid TS** See diazobenzenesulfonic acid TS, concentrated.

**Congo red**  $C_{32}H_{22}N_6Na_2O_6S_2$  [K 8352, Special class]

**Congo red TS** Dissolve 0.5 g of congo red in 100 mL of a mixture of ethanol (95) and water (1:9).

**Control anti-interleukin-2 antiserum TS** Anti-interleukin-2 antiserum is diluted with culture media for celmoleukin, so that the diluted antiserum solution neutralizes the same volume of about 800 unit/mL solution of Celmoleukin (Genetical Recombination).

**Coomassie brilliant blue G-250**  $C_{47}H_{48}N_3NaO_7S_2$  A deep violet powder. A solution in ethanol (99.5) (1 in 100,000) exhibits an absorption maxima at a wavelength of 608 nm.

**Coomassie brilliant blue R-250**  $C_{45}H_{44}N_3NaO_7S_2$  Deep blue-purple powder. Odorless.  
*Content*: not less than 50%.

**Coomassie staining TS** Dissolve 125 mg of Coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

**Copper** Cu [K 8660, Special class]

**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-acetone TS** Dissolve 0.3 g of copper (II) chloride dihydrate in acetone to make 10 mL.

**Copper (II) chloride dihydrate**  $CuCl_2 \cdot 2H_2O$  [K 8145, Special class]

**Copper (II) disodium ethylenediamine tetraacetate tetrahydrate**  $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$  A blue powder.

*pH* <2.54>: 7.0–9.0

*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 100 mL. Pipet 10 mL of this solution, adjust the pH of the mixture to about 1.5 by adding 100 mL of water and dilute nitric acid, then add 5 mL of a solution of 1,10-phenanthroline mono-

hydrate in methanol (1 in 20), and titrate <2.50> 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).

Each mL 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, and 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 <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the liquid is changed from yellow-green to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS  
= 0.9756 mg of  $Cu(OH)_2$

**Copper (II) sulfate**  $CuSO_4$  [K 8984, First class]

**Copper (II) sulfate pentahydrate**  $CuSO_4 \cdot 5H_2O$  [K 8983, Special class]

**Copper (II) sulfate-pyridine TS** Dissolve 4 g of copper (II) sulfate pentahydrate in 90 mL of water, then add 30 mL of pyridine. Prepare before use.

**Copper (II) sulfate TS, 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 1000 mL.

**Copper (II) sulfate TS** Dissolve 12.5 g of copper (II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L).

**Copper (standard reagent)**  $Cu$  [K 8005, Standard reagent for quantitative analysis]

**Coptisine chloride for thin-layer chromatography**

$C_{19}H_{14}NO_4Cl$  Orange-red, crystals or crystalline powder. Slightly soluble in methanol, and very slightly soluble in water and in ethanol (99.5). **Melting point:** about 260°C (with decomposition).

**Identification** Determine the absorption spectrum of a solution (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 237 nm and 241 nm, between 264 nm and 268 nm, between 354 nm and 358 nm, and between 452 nm and 462 nm.

**Purity** Related substances—Dissolve 1 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1  $\mu L$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spot other than the principal spot (*R<sub>f</sub>* value is about 0.4) obtained from the sample solution is not more in-

tense than the spot from the standard solution.

**Corn oil** [Same as the namesake monograph]

**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* Linné (*Gossypium*) or of other similar species. A pale yellow, odorless, oily liquid. Miscible with diethyl ether, and with hexane. Slightly soluble in ethanol (95).

**Refractive index** <2.45>  $n_D^{20}$ : 1.472 – 1.474

**Specific gravity** <2.56>  $d_{25}^{25}$ : 0.915 – 0.921

**Acid value** <1.13>: not more than 0.5.

**Saponification value** <1.13>: 190 – 198

**Iodine value** <1.13>: 103 – 116

**Cresol**  $CH_3C_6H_4(OH)$  [Same as the namesake monograph]

**m-Cresol**  $CH_3C_6H_4(OH)$  [K 8305, Special class]

**m-Cresol purple** See metacresol purple.

**m-Cresol purple TS** See metacresol purple TS.

**p-Cresol**  $C_7H_8O$  [K 8306, Special class]

**Cresol red**  $C_{21}H_{18}O_5S$  [K 8308, Special class]

**Cresol red TS** Dissolve 0.1 g of cresol red in 100 mL of ethanol (95), and filter if necessary.

**Crystalline 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. Assay—(i) Sample solution: Weigh accurately an appropriate amount of crystallized trypsin according to the labeled Units, dissolve in 0.001 mol/L hydrochloric acid TS to prepare a solution containing 50 FIP Units per mL, and use this solution as the sample solution. Prepare before use, and preserve in ice. (ii) 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-silver chloride electrode for pH determination, nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature at  $25 \pm 0.1^\circ C$  by means of a precise thermoregulator. (iii) Procedure: Pipet 1.0 mL of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester TS, transfer to the reaction reservoir, and add 9.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0. Allow to stand in the thermostat for 10 minutes to make the temperature of the contents reach to  $25 \pm 0.1^\circ C$ , 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 0.05 mL of the sample solution previously allowed to stand at  $25 \pm 0.1^\circ C$ , then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50  $\mu L$ -micropipet (minimum graduation of 1  $\mu L$ ) while stirring to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 8 minutes. Separately, transfer 10 mL of sodium

tetraborate-calcium chloride buffer solution, pH 8.0, and perform a blank determination in the same manner. (iv) Calculation: Plot the amount of consumption ( $\mu\text{L}$ ) of 0.1 mol/L sodium 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  $\mu\text{mol}$  of sodium hydroxide consumed per minute as  $D$  (FIP Unit).

$$D (\mu\text{mol NaOH/min}) = \frac{v_2 - v_1}{t_2 - t_1} \times f \times \frac{1}{10}$$

$f$ : Factor of 0.1 mol/L sodium hydroxide VS

FIP Units per mg of crystallized trypsin to be tested

$$= \frac{(D_1 - D_0) \times T}{L \times M}$$

$D_1$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the sample solution is used

$D_0$ :  $\mu\text{mol}$  of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

$M$ : Amount (mg) of crystallized trypsin sampled

$L$ : Amount (mL) of the sample solution put in the reaction reservoir

$T$ : Total volume (mL) of the sample solution prepared by dissolving in 0.001 mol/L hydrochloric acid TS

One FIP Unit is an amount of enzyme which decomposes 1  $\mu\text{mol}$  of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester per minute under the conditions described in the Assay.

*Storage*—Preserve in a cold place.

**Crystalline trypsin for ulinastatin assay** A proteolytic enzyme prepared from bovine pancreas. White to light yellow crystalline powder. Odorless. Sparingly soluble in water, and dissolves in 0.001 mol/L hydrochloric acid TS.

*Content*: not less than 3200 trypsin Units per mg. Assay—(i) Sample solution: Weigh accurately about 20 mg of crystalline trypsin for ulinastatin assay, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 3000 trypsin Units. Dilute this solution with 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 40 trypsin Units, and use this solution as the sample solution. (ii) Diluent: Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6. (iii) Substrate solution: Dissolve 85.7 mg of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the stock solution, add the diluent to make exactly 100 mL, and use this solution as the substrate solution. The absorbance of the substrate solution determined at 253 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank is between 0.575 and 0.585. If the absorbance of the substrate solution is not in this range, adjust with the diluent or the substrate stock solution. (iv) Procedure: Pipet 3 mL of the substrate solution, previously warmed at  $25 \pm 0.1^\circ\text{C}$ , into a 1-cm quartz cell, add exactly 0.2 mL of the sample solution, and start the determination of the absorbance change at 253 nm for 5 minutes at  $25 \pm 0.1^\circ\text{C}$  using a solution prepared by adding exactly 0.2 mL of 0.001 mol/L hydrochloric acid TS to exactly 3 mL of the substrate solution as the

blank. Determine the difference of the absorbance change per minute,  $A$ , when the difference has been constant for at least 3 minutes. (v) Calculation: Trypsin Units per mg is obtained by use of the following equation. One trypsin Unit is an amount of the enzyme which gives 0.003 change in absorbance per minute under the conditions described above.

$$\text{Trypsin Units per mg} = \frac{A}{0.003 \times M}$$

$M$ : Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

*Storage*—Preserve in a cold place.

**Crystal violet**  $\text{C}_{25}\text{H}_{30}\text{CN}_3 \cdot 9\text{H}_2\text{O}$  [K 8294, Special class]

**Crystal violet TS** Dissolve 0.1 g of crystal violet 10 mL of acetic acid (100).

**Culture medium for assay of teceleukin** Add 100 mL of fetal calf serum to 1000 mL of medium for float culture. Store at  $4^\circ\text{C}$ .

**Culture medium for celmoleukin** Take a specified amount of RPMI-1640 powdered medium that contains glutamate but does not contain sodium hydrogen carbonate, add water to dissolve, and add *N*-2-hydroxyethylpiperidine-*N*-2-ethansulfonic acid as a buffering agent to a concentration of 0.025 mol/L. To 1000 mL of this solution add 0.1 g (potency) of streptomycin sulfate, 100,000 units of potassium benzylpenicillin, and 2 g of sodium hydrogen carbonate, adjust the pH to 7.1 to 7.2 with sodium hydroxide TS, and then sterilize by filtration. To this solution add fetal calf serum heated at  $56^\circ\text{C}$  for 30 minutes to 20 vol%.

**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 50 mL. 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 Ultraviolet-visible Spectrophotometry <2.24>, 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**  $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$  [K 8289, 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**  $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$   
A blue to blue-green powder. It is insoluble in water, and dissolves foamingly in dilute acid. It dissolves in ammonia TS and shows a deep blue color.

*Purity* (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: not more than 0.120%.

(3) Iron—Dissolve 5.0 g of cupric carbonate monohydrate 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-acetone TS** See copper (II) chloride-acetone TS.

**Cupric sulfate** See copper (II) sulfate pentahydrate.

**Cupric sulfate, anhydrous** See copper (II) sulfate (anhydrous).

**Cupric sulfate-pyridine TS** See copper (II) sulfate-pyridine TS.

**Cupric sulfate solution, alkaline** See copper (II) sulfate solution, alkaline.

**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 1.96–2.04, Y is 0.98–1.02 and X/Y is 1.96–2.04 by adding water, copper (II) hydroxide or ethylenediamine 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 ( $V_1$ ) of the solution to be assayed, add 60 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (pH Determination <2.54>); End point is about pH 8.4).

$$X = \frac{N_1 a}{V_1}$$

X: Concentration of ethylenediamine (mol/L)

a: Volume of 0.1 mol/L hydrochloric acid VS consumed for the titration (mL)

$N_1$ : Concentration of 0.1 mol/L hydrochloric acid VS (mol/L)

(2) Copper (II) ion—Pipet 2 mL ( $V_2$ ) of the solution to be assayed, add 20 mL of water, about 3 g of potassium

iodide and 50 mL of 2 mol/L sulfuric acid TS, shake for 5 minutes, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution turns light 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 then titrate 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)

$N_2$ : Concentration of 0.1 mol/L sodium thiosulfate VS (mol/L)

**Curcumin**  $C_{21}H_{20}O_6$  A reddish yellow crystalline powder.

**Melting point** <2.60>: 180 – 183°C.

Preserve in a light-resistant tight container.

**Curcumin TS** Dissolve 0.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare before use.

**Curcumin for assay**  $C_{21}H_{20}O_6$  Yellow to orange crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (422 nm): 1460 – 1700 (dried for 24 hours in a desiccator (in vacuum, silica gel), 2.5 mg, methanol, 1000 mL).

**Melting point** <2.60>: 180 – 184°C

**Purity** Related substances—(1) Dissolve 4 mg of curcumin for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 5  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the  $R_f$  value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of curcumin for assay in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of curcumin obtained from the sample solution is not larger than the peak area of curcumin from the standard solution. Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Turmeric.

Detector: A visible absorption photometer (wavelength: 422 nm).

Time span of measurement: About 4 times as long as the retention time of curcumin beginning after the solvent peak. System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add methanol to make exactly 20 mL. Confirm that the peak area of curcumin obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of curcumin from 10  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Turmeric.

**Curcumin for component determination** See curcumin for assay.

**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 <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 85.06 mg of  $C_3H_3NO_2$

**Cyanocobalamin**  $C_{63}H_{88}CoN_{14}O_{14}P$  [Same as the namesake monograph]

**Cyanogen bromide TS** To 100 mL of ice-cold water add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide TS dropwise 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.

**1-Cyanoguanidine**  $NH_2C(NH)NHCN$  A white crystalline powder. Freely soluble in water.

*Melting point* <2.60>: 209 – 212°C

*Loss on drying* <2.41>: not more than 0.1% (1 g, 105°C, 3 hours).

*Nitrogen content* <1.08>: 66.0 – 67.3% (after drying).

**Cyanopropylmethylphenylsilicone for gas chromatography** Prepared for gas chromatography.

**6% Cyanopropylphenyl-94% dimethyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**6% Cyanopropyl-6% phenyl-methyl silicone polymer for gas chromatography** Prepared for gas chromatography.

**7% Cyanopropyl-7% phenylmethylsilicone polymer for gas chromatography** Prepared for gas chromatography.

**Cycloartenyl ferulate for thin-layer chromatography**  $C_{40}H_{58}O_4$  A white to light brown, crystalline powder or powder. Soluble in acetone, slightly soluble in acetonitrile, and practically insoluble in water and in methanol. Melting point: about 155°C.

*Identification* (1) Determine the absorption spectrum of a solution in heptane (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 229 nm and 233 nm, between 289 nm and 293 nm, and between 313 nm and 317 nm.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940  $cm^{-1}$ , 1691  $cm^{-1}$ , 1511  $cm^{-1}$  and

1270  $cm^{-1}$ .

*Purity* Related substances—Dissolve 2.0 mg in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 5  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Brown Rice: the spot other than the principle spot, having Rf value of about 0.4, obtained from the sample solution is not more intense than the spot from the standard solution.

**Cyclohexane**  $C_6H_{12}$  [K 8464, Special class]

**Cyclohexylamine**  $C_6H_{11}NH_2$  A clear and colorless liquid, having a characteristic amine-like odor. Miscible with water, with *N,N*-dimethylformamide and with acetone.

*Purity* Related substances—Use cyclohexylamine as the sample solution. Separately, pipet 1 mL of cyclohexylamine, add hexane to make exactly 100 mL, and use this as the standard solution. Perform the test as directed in Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol, ammonia water (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

**Cyclohexylmethanol**  $C_7H_{14}O$  A liquid having slight camphor odor. Soluble in ethanol (99.5).

*Refractive index* <2.45>  $n_D^{20}$ : about 1.464

*Boiling point* <2.57>: about 185°C

**Cyclosporine U**  $C_{81}H_{109}N_{11}O_{12}$  White powder.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : about  $-190^\circ$  (0.1 g, methanol, 20 mL 100 mm).

**L-Cysteic acid**  $C_3H_7NO_5S$  White powder.

*Melting point* <2.60>: about 260°C.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ :  $+7.5 - +9.0^\circ$  (1.5 g, water, 20 mL, 100 mm).

**L-Cysteine hydrochloride** See L-cysteine hydrochloride monohydrate.

**L-Cysteine hydrochloride monohydrate**  $HSCH_2CH(NH_2)COOH.HCl.H_2O$  [K 8470, Special class]

**L-Cystine**  $HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$  [K 9048, L(-)-Cystine, Special class]

**Cytochrome c** An oxidase (molecular weight: 8000 – 13,000) derived from bovine cardiac muscle.

**Cytosine**  $C_4H_5N_3O$  White, crystalline powder or powder.

*Absorbance* <2.24>  $E_{1\%}^{1\text{cm}}$  (276 nm): not less than 800 (after drying, 40 mg, 10,000 mL of 0.1 mol/L hydrochloric acid TS).

**Dacurionium Bromide for thin-layer chromatography**  $C_{33}H_{58}Br_2N_2O_3$  White crystalline powder. Very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetic anhydride. Hygroscopic.

*Identification*—Determine the infrared absorption spectrum of dacurionium bromide for thin-layer chromatography

according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits the absorptions at the wave numbers at about 2940  $\text{cm}^{-1}$ , 1737  $\text{cm}^{-1}$ , 1630  $\text{cm}^{-1}$ , 1373  $\text{cm}^{-1}$ , 1233  $\text{cm}^{-1}$  and 1031  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 10 mg of dacrurionium bromide for thin-layer chromatography in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Purity (2) Related substances under Pancuronium Bromide: the spots other than the principal spot from the sample solution do not show more intense color than the spot from the standard solution.

**Water** <2.48>: not more than 1.0% (1 g, volumetric titration, direct titration).

**Content:** not less than 98.0%, calculated on the dehydrated basis. **Assay**—Weigh accurately about 0.2 g of dacrurionium bromide for thin-layer chromatography, dissolve in 50 mL of acetic anhydride by warming, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 34.53 mg of  $\text{C}_{33}\text{H}_{58}\text{Br}_2\text{N}_2\text{O}_3$

***p,p'*-DDD** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane)  
 $\text{C}_{14}\text{H}_{10}\text{Cl}_4$

**Melting point** <2.60>: 108 – 110°C

**Purity** Related substances—Dissolve 10 mg of *p,p'*-DDD in hexane for purity of crude drug to make exactly 100 mL, pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than *p,p'*-DDD from the sample solution is not larger than the peak area of *p,p'*-DDD from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

**Detection sensitivity:** Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of *p,p'*-DDD obtained from 1  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of *p,p'*-DDD from 1  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About twice as long as the retention time of *p,p'*-DDD beginning after the solvent peak.

***p,p'*-DDE** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene)  $\text{C}_{14}\text{H}_8\text{Cl}_4$

**Melting point** <2.60>: 88 – 90°C

**Purity** Related substances—Proceed as directed in the

Purity of *p,p'*-DDD using the following standard solution (1).

**Standard solution (1):** Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

***o,p'*-DDT** (1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane)  $\text{C}_{14}\text{H}_9\text{Cl}_5$

**Melting point** <2.60>: 73 – 75°C

**Purity** Related substances—Proceed as directed in the Purity of *p,p'*-DDD.

***p,p'*-DDT** (1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane)  $\text{C}_{14}\text{H}_9\text{Cl}_5$

**Melting point** <2.60>: 108 – 110°C

**Purity** Related substances—Proceed as directed in the Purity of *p,p'*-DDD using the following standard solution (1).

**Standard solution (1):** Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

**Decolorized fuchsin TS** Add 1 g of fuchsin in 100 mL of water, heat at about 50°C, then cool with occasional shaking. After standing for 48 hours, mix and filter. To 4 mL of the filtration add 6 mL of hydrochloric acid and water to make 100 mL. Use after standing for at least 1 hour. Prepare before use.

***n*-Decyl trimethylammonium bromide**  $\text{C}_{13}\text{H}_{30}\text{NBr}$

White powder. **Melting point:** about 232°C (with decomposition).

**Content:** not less than 99%. **Assay**—Weigh accurately about 0.5 g of *n*-decyl trimethylammonium bromide, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 28.03 mg of  $\text{C}_{13}\text{H}_{30}\text{NBr}$

**0.005 mol/L *n*-Decyl trimethylammonium bromide TS**

Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.40 g of *n*-decyl trimethylammonium bromide in water to make 1000 mL.

**Defibrinated blood of rabbit** Transfer 100 mL of blood obtained from rabbit to a flask, put in about 20 glass balls 8 mm in diameter, shake for 5 minutes gently, and filter through gauze. Prepare before use.

**Dehydrated ethanol** See ethanol (99.5).

**Dehydrated ether** See diethyl ether.

**Dehydrated pyridine** See pyridine, dehydrated.

**Dehydrocorydaline nitrate for assay**  $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_7$  Yellow, crystals or crystalline powder. It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). **Melting point:** about 240°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (333 nm): 577 – 642 (3 mg, water, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the test.

**Purity** (1) Related substances 1—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 1 mL of a mixture of

water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Spray Dragendorff's TS on the plate, air-dry, and spray sodium nitrite TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(2) Related substances 2—Dissolve 5.0 mg of dehydrocorydaline nitrate for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of peaks other than dehydrocorydaline from the sample solution is not larger than the peak area of dehydrocorydaline from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under *Corydalis Tuber*.

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

Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline, beginning after the peak of nitric acid.

#### System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under *Corydalis Tuber*.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained from 5  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that from 5  $\mu$ L of the standard solution.

#### Dehydrocorydaline nitrate for component determination

See dehydrocorydaline nitrate for assay.

**Demethoxycurcumin**  $C_{20}H_{18}O_5$  Yellow to orange crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 166 – 170°C.

*Identification* Determine the absorption spectrum of a solution of demethoxycurcumin in methanol (1 in 400,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 416 nm and 420 nm.

*Purity* Related substances—(1) Dissolve 4 mg of demethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for

thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the *R<sub>f</sub>* value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of demethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of demethoxycurcumin obtained from the sample solution is not larger than the peak area of demethoxycurcumin from the standard solution.

#### Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under *Turmeric*.

Detector: A visible absorption photometer (wavelength: 422 nm).

Time span of measurement: About 4 times as long as the retention time of demethoxycurcumin beginning after the solvent peak.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of demethoxycurcumin obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of demethoxycurcumin from 10  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under *Turmeric*.

***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 <2.25> 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**  $C_9H_{12}N_2O_5$  White, crystalline powder.

*Melting point* <2.60>: 162 – 166°C

*Purity*—Dissolve 3.0 mg of 2'-deoxyuridine for liquid chromatography in diluted methanol (1 in 25) to make 50 mL. Perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Purity under *Idoxuridine Ophthalmic Solution*. Measure each peak area by the automatic integration method to the range about twice the retention time of 2'-deoxyuridine, and calculate the amount of 2'-deoxyuridine by the area percentage method: it shows a purity of not less than 98.5%.

**Content:** not less than 98.5%. **Assay**—Weigh accurately about 5 mg of 2'-deoxyuridine for liquid chromatography, previously dried in vacuum at 60°C for 3 hours, and dissolve in water to make exactly 250 mL. Pipet 10 mL of this solution, dilute with water to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine absorbance *A* at the maximum wavelength at about 262 nm.

$$\begin{aligned} & \text{Amount (mg) of deoxyuridine (C}_9\text{H}_{12}\text{N}_2\text{O}_5\text{)} \\ &= \frac{A}{447} \times 5000 \end{aligned}$$

**Dermatan sulfate** Dermatan sulfate is mucopolysaccharide purified from the skin and small intestines of pigs by alkaline extraction, followed by digestion with protease and fractionation by alcohol. When cellulose acetate membrane electrophoresis of dermatan sulfate is performed and the membrane is stained in a toluidine blue O solution (1 in 200), a single band appears.

Operation conditions of cellulose acetate membrane electrophoresis—

Cellulose acetate membrane: 6 cm in width and 10 cm in length.

Mobile phase: Dissolve 52.85 g of calcium acetate monohydrate in water to make 100 mL.

Run time: 3 hours (1.0 mA/cm)

**Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy** (CD<sub>3</sub>)<sub>2</sub>SO Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated formic acid for nuclear magnetic resonance spectroscopy** DCOOD Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy** DCl Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated methanol for nuclear magnetic resonance spectroscopy** CD<sub>3</sub>OD Prepared for nuclear magnetic resonance spectroscopy.

**Deuterated NMR solvents** Prepared for nuclear magnetic resonance spectroscopy. For example: deuterated dimethylsulfoxide [(CD<sub>3</sub>)<sub>2</sub>SO], deuterated pyridine (C<sub>5</sub>D<sub>5</sub>N), deuteriochloroform (CDCl<sub>3</sub>), heavy water (D<sub>2</sub>O), etc.

**Deuterated pyridine for nuclear magnetic resonance spectroscopy** C<sub>5</sub>D<sub>5</sub>N Prepared for nuclear magnetic resonance spectroscopy.

**Deuteriochloroform for nuclear magnetic resonance spectroscopy** CDCl<sub>3</sub> Prepared for nuclear magnetic resonance spectroscopy.

**Devarda's alloy** [K 8653, For Nitrogen analysis]

**Diacetyl** CH<sub>3</sub>COCOCH<sub>3</sub> 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** <2.42>: -2.0 - -5.5°C

**Refractive index** <2.45> *n*<sub>D</sub><sup>20</sup>: 1.390 - 1.398

**Specific gravity** <2.56> *d*<sub>20</sub><sup>20</sup>: 0.98 - 1.00

**Boiling point** <2.57>: 85 - 91°C

**Purity** Clarity of solution—Dissolve 1.0 g of diacetyl in

10 mL 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 <2.50> 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.

$$\begin{aligned} & \text{Each mL of 0.5 mol/L hydrochloric acid VS} \\ &= 21.52 \text{ mg of C}_4\text{H}_6\text{O}_2 \end{aligned}$$

**Diacetyl TS** Dissolve 1 mL of diacetyl in water to make 100 mL, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

**2,3-Diaminonaphthalene** C<sub>10</sub>H<sub>10</sub>N<sub>2</sub> Light yellow-brown crystals or powder. Slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Melting point** <2.60>: 193 - 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-diaminonaphthalene 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 cyclohexane by thorough shaking for 2 minutes, and centrifuge the cyclohexane layers to remove moisture. When the absorbance 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 Ultraviolet-visible Spectrophotometry <2.24>, it is not less than 0.08.

**Selenium standard solution**—Weigh accurately 40 mg 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 5 mL 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) per mL.

**2,4-Diaminophenol dihydrochloride** C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O.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** <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44>: 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 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L silver nitrate VS} \\ &= 9.853 \text{ mg of C}_6\text{H}_8\text{N}_2\text{O.2HCl} \end{aligned}$$

**2,4-Diaminophenol dihydrochloride TS** Dissolve 1 g of 2,4-diaminophenol hydrochloride and 20 g of sodium bisulfite in 100 mL of water, and filter, if necessary.

**2,4-Diaminophenol hydrochloride** See 2,4-diaminophenol dihydrochloride.

**2,4-Diaminophenol hydrochloride TS** See 2,4-diaminophenol dihydrochloride TS.

**Diammonium hydrogen citrate**  $C_6H_{14}N_2O_7$  [K 8284, Special class]

**Diammonium hydrogen phosphate**  $(NH_4)_2HPO_4$  [K 9016, Special class]

**Diazepam for assay**  $C_{16}H_{13}ClN_2O$  [Same as the monograph, Diazepam. When dried, it contains not less than 99.0% of diazepam ( $C_{16}H_{13}ClN_2O$ ), and meets the additional following requirement.]

**Purity** Related substance—Dissolve 50 mg in 10 mL of water, add methanol to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than diazepam from the sample solution is not larger than the peak area of diazepam from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Diazepam Tablets.

Time span of measurement: About 4.5 times as long as the retention time of diazepam, beginning after the solvent peak. System suitability

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diazepam are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diazepam is not more than 2.0%.

**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 100 mL. 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) dropwise 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 50 mL. Store in a cold place, and use within 8 hours.

**Dibasic ammonium phosphate** See diammonium hydrogen phosphate.

**Dibasic potassium phosphate** See dipotassium hydrogen phosphate.

**Dibasic potassium phosphate-citric acid buffer solution, pH 5.3** See dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3.

**1 mol/L Dibasic potassium phosphate TS for buffer solution** See 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

**Dibasic sodium ammonium phosphate** See ammonium sodium hydrogen phosphate tetrahydrate.

**Dibasic sodium phosphate** See disodium hydrogen phosphate dodecahydrate.

**Dibasic sodium phosphate, anhydrous** See disodium hydrogen phosphate.

**Dibasic sodium phosphate, anhydrous, for pH determination** See disodium hydrogen phosphate for pH determination.

**Dibasic sodium phosphate-citric acid buffer solution, pH 4.5** See disodium hydrogen phosphate-citric acid buffer solution, pH 4.5.

**Dibasic sodium phosphate-citric acid buffer solution, pH 5.4** See disodium hydrogen phosphate-citric acid buffer solution, pH 5.4.

**Dibasic sodium phosphate-citric acid buffer solution, pH 6.0** See disodium hydrogen phosphate-citric acid buffer solution, pH 6.0.

**Dibasic sodium phosphate TS** See disodium hydrogen phosphate TS.

**0.05 mol/L Dibasic sodium phosphate TS** See 0.05 mol/L disodium hydrogen phosphate TS.

**0.5 mol/L Dibasic sodium phosphate TS** See 0.5 mol/L disodium hydrogen phosphate TS.

**Dibekacin sulfate**  $C_{18}H_{37}N_5O_8 \cdot xH_2SO_4$  [Same as the namesake monograph]

**Dibenz[*a,h*]anthracene**  $C_{22}H_{14}$  Very pale yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 265 – 270°C.

**Identification** Perform the test with dibenz[*a,h*]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak ( $m/z$  278) and a fragment ion peak ( $m/z$  139).

**Purity** Related substances—Dissolve 3.0 mg of dibenz[*a,h*]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1

$\mu\text{L}$  of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than dibenz[*a,h*]anthracene is not more than 7.0%.

Operating conditions

Detector: A mass spectrophotometer (EI).

Mass scan range: 15.00 – 300.00.

Time of measurement: 12 – 30 minutes.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 – 0.5  $\mu\text{m}$ .

Column temperature: Inject at a constant temperature of about 45°C, raise the temperature to 240°C at a rate of 40°C per minute, maintain at 240°C for 5 minutes, raise to 300°C at a rate of 4°C per minute, raise to 320°C at a rate of 10°C per minute, and maintain at 320°C for 3 minutes.

Injection port temperature: A constant temperature of about 250°C.

Interface temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the reaction time of the peak of dibenz[*a,h*]anthracene is about 27 minutes.

Split ratio: Splitless.

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of dibenz[*a,h*]anthracene obtained from 1  $\mu\text{L}$  of this solution is equivalent to 5 to 15% of that of dibenz[*a,h*]anthracene from 1  $\mu\text{L}$  of the standard solution.

**Dibenzyl**  $\text{C}_{14}\text{H}_{14}$  White crystals, freely soluble in diethyl ether, soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point <2.60> 50 – 54°C

**Purity** Related substances—Dissolve 32 mg of dibenzyl in methanol to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 20  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Assay under Vinblastine Sulfate for Injection: any peak other than the principal peak does not appear. Adjust the detection sensitivity so that the peak height of dibenzyl obtained from 20  $\mu\text{L}$  of the solution prepared by adding methanol to 10 mL of the sample solution to make 20 mL, is 3 to 5 cm, and the time span of measurement is about 1.2 times as long as the retention time of dibenzyl after the solvent peak.

***N,N'*-Dibenzylethylenediamine diacetate**

$\text{C}_{16}\text{H}_{20}\text{N}_2 \cdot 2\text{C}_2\text{H}_4\text{O}_2$  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 <2.25>: it exhibits absorption at the wave numbers of about 1530  $\text{cm}^{-1}$ , 1490  $\text{cm}^{-1}$ , 1460  $\text{cm}^{-1}$ , 1400  $\text{cm}^{-1}$  and 1290  $\text{cm}^{-1}$ .

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 25 mg of *N,N'*-dibenzylethylenediamine diacetate, dissolve in 25 mL 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 5 mL 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 25 mL of methanol, and add the 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 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  $\mu\text{L}$  each of the sample solution and control solution as directed under Liquid Chromatography <2.01> 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 variance 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 silica gel for liquid chromatography (5  $\mu\text{m}$  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 Benzylpenicillin 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 5 mL 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  $\mu\text{L}$  of this solution under the above operating conditions, *N,N'*-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

**System repeatability:** When the test is repeated 6 times with 20  $\mu\text{L}$  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-benzoquinone monoimine**  
 $\text{C}_6\text{H}_2\text{Br}_2\text{ClNO}$  [K 8491, Special class]

**2,6-Dibromo-*N*-chloro-1,4-benzoquinone monoimine TS**  
Dissolve 0.5 g of 2,6-dibromo-*N*-chloro-1,4-benzoquinone

monoimine in methanol to make 100 mL.

**2,6-Dibromo-*N*-chloro-1,4-benzoquinone monoimine TS, dilute** Dissolve 0.2 g of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

**2,6-Dibromoquinone chlorimide** See 2,6-dibromo-*N*-chloro-1, 4-benzoquinone monoimine.

**2,6-Dibromoquinone chlorimide TS** See 2,6-dibromo-*N*-chloro-1, 4-benzoquinone monoimine TS.

**Dibucaine hydrochloride** C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>2</sub>·HCl [Same as the namesake monograph]

**Dibutylamine** C<sub>8</sub>H<sub>19</sub>N Colorless, clear liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.415 – 1.419

*Density* <2.56> (20°C): 0.756 – 0.761 g/mL

**Di-*n*-butyl ether** (C<sub>4</sub>H<sub>9</sub>)<sub>2</sub>O Clear colorless, water-non-miscible liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.768 – 0.771

**Di-*n*-butyl phthalate** C<sub>6</sub>H<sub>4</sub>(COOC<sub>4</sub>H<sub>9</sub>)<sub>2</sub> Clear colorless liquid.

*Purity* Related substances—Dissolve 0.5 g of di-*n*-butyl phthalate in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Nicardipine Hydrochloride Injection, and determine the peak area by the automatic integration method. Calculate the amount of di-*n*-butyl phthalate by the area percentage method: the amount of di-*n*-butyl phthalate is not less than 98.0%, and no peak appears at the same position as nicardipine. Adjust the detection sensitivity so that the peak height of di-*n*-butyl phthalate obtained from 10 μL of the sample solution is 50 to 100% of the full scale, and measure about 2 times as long as the retention time of di-*n*-butyl phthalate after the solvent peak.

**1,2-Dichlorobenzene** C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub> A colorless liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.306

*Boiling point* <2.57>: 180 – 181°C

**1,2-Dichloroethane** ClCH<sub>2</sub>CH<sub>2</sub>Cl [K 8465, Special class]

**Dichlorofluorescein** C<sub>20</sub>H<sub>10</sub>Cl<sub>2</sub>O<sub>5</sub> Orange to red-brown powder.

*Identification* (1) Dissolve 0.1 g in 10 mL of sodium hydroxide TS: the solution is an orange-red color, and red-orange precipitates appear by the addition of 10 mL of dilute hydrochloric acid.

(2) Dissolve 0.1 g in 10 mL 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 dihydrate** C<sub>12</sub>H<sub>6</sub>Cl<sub>2</sub>NNaO<sub>2</sub>·2H<sub>2</sub>O [K 8469, 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.

**2,6-Dichloroindophenol sodium-sodium acetate TS** Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

**Dichloromethane** CH<sub>2</sub>Cl<sub>2</sub> [K 8161, Special class]

**2,6-Dichlorophenol** C<sub>6</sub>H<sub>4</sub>Cl<sub>2</sub>O White to purplish white crystals.

*Melting point* <2.60>: 65 – 67°C

**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-indophenol sodium TS for titration** See 2,6-dichloroindophenol sodium TS for titration.

**Dicyclohexyl** C<sub>12</sub>H<sub>22</sub>

*Specific gravity* <2.56>  $d_{20}^{20}$ : about 0.864

*Boiling point* <2.57>: about 227°C

*Melting point* <2.60>: about 4°C

***N,N'*-Dicyclohexylcarbodiimide** C<sub>13</sub>H<sub>22</sub>N<sub>2</sub> Colorless or white crystals or crystalline mass. Dissolves in ethanol (95), but decomposes in water to produce a white precipitate.

*Melting point* <2.60>: 35 – 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) to make 100 mL.

*Storage*—Preserve in tight containers, in a cold place.

**Dicyclohexyl phthalate** C<sub>6</sub>H<sub>4</sub>(COOC<sub>6</sub>H<sub>11</sub>)<sub>2</sub> A white, crystalline powder.

*Melting point* <2.60>: 63 – 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.

**Dicyclohexylurea** C<sub>6</sub>H<sub>11</sub>NHCONHC<sub>6</sub>H<sub>11</sub> A white crystalline powder, having no odor.

*Purity* Related substances—Dissolve 50 mg of dicyclohexylurea in methanol to make 100 mL. Pipet 10 mL of this solution, and add methanol to make 100 mL. Pipet 20 mL of this solution, add 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add 5 mL of diluted hydrochloric acid (1 in 10), and shake. Perform the test with 50 μL of this solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the area of each peak by the automatic integration method, and calculate the amount by the area percentage method: the total amount of the peaks other than dicyclohexylurea is not more than 3.0%.

*Operating conditions*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) (ii) under Acetohexamide.

Time span of measurement: About 5 times as long as the retention time of dicyclohexylurea beginning after the solvent peak.

System suitability

Test for required detectability: To exactly 5 mL of the

standard solution add water to make exactly 200 mL. Confirm that the peak area of dicyclohexylurea obtained with 50  $\mu$ L of this solution is equivalent to 1.8 to 3.3% of that with 50  $\mu$ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Purity (4) (ii) under Acetohexamide.

**Diethanolamine**  $C_4H_{11}NO_2$  Colorless viscous liquid.

Melting point <2.60>: 27 – 30°C

Water <2.48>: less than 0.1%.

**Diethanolamine hydrochloride** See 2,2'-iminodiethanol hydrochloride.

**Diethylamine**  $(C_2H_5)_2NH$  A clear, colorless liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline, and readily absorbs carbon dioxide in air.

Specific gravity <2.56>  $d_4^{10}$ : 0.702 – 0.708

Distilling range <2.57>: 54 – 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 <2.50> the excess of sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination.

Each mL 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 and with ethanol (95).

Specific gravity <2.56>  $d_{20}^{20}$ : 1.118 – 1.120

**Diethylene glycol adipinate for gas chromatography** Prepared for gas chromatography.

**Diethylene glycol dimethyl ether**  $(CH_3OCH_2CH_2)_2O$

Clear and colorless liquid, miscible with water.

Specific gravity <2.56>  $d_4^{20}$ : 0.940 – 0.950

Distilling range <2.57>: 158 – 160°C, not less than 95 vol%.

**Diethylene glycol monoethyl ether**  $C_2H_5(OCH_2CH_2)_2OH$  [2-(2-ethoxyethoxy)ethanol] Clear, colorless liquid, of which boiling point is about 203°C. It freely mixed with water.

Refractive index <2.45>  $n_D^{20}$ : 1.425 – 1.429

Specific gravity <2.56>  $d_{20}^{20}$ : 0.990 – 0.995

Acid (as  $CH_3COOH$ ): less than 0.01%.

**Diethylene glycol monoethyl ether for water determination** See Water Determination <2.48>.

**Diethylene glycol succinate ester for gas chromatography** Prepared for gas chromatography.

**Diethylene glycol succinate polyester for gas chromatography** Prepared for gas chromatography.

**Diethyl ether**  $C_2H_5OC_2H_5$  [K 8103, Special class]

**Diethyl ether, dehydrated**  $C_2H_5OC_2H_5$  [K 8103, Special class. The water content is not more than 0.01%.]

**Diethyl ether for purity of crude drug**  $C_2H_5OC_2H_5$  [K 8103, Special class] Use diethyl ether meeting the following additional specification. Evaporate 300.0 mL of diethyl ether for purity of crude drug in vacuum at a temperature not higher than 40°C, add the diethyl ether to make

exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with 1  $\mu$ L each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of  $\gamma$ -BHC from the standard solution I.

Operating conditions

Proceed the operating conditions in the Purity (2) under the Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained from 1  $\mu$ L of the standard solution II can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC from 1  $\mu$ L of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of  $\gamma$ -BHC beginning after the peak of solvent.

***N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate**

$C_{18}H_{24}N_2O_4$  A white crystalline powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: 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-naphthylethylenediamine oxalate-acetone TS** Dissolve 1 g of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate in 100 mL of a mixture of acetone and water (1:1). Prepare before use.

***N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate TS** Dissolve 1 g of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate in water to make 1000 mL.

**Diethyl phthalate**  $C_6H_4(COOC_2H_5)_2$  A colorless, clear liquid.

Refractive index <2.45>  $n_D^{20}$ : 1.500 – 1.505

Purity Related substances—To 1 mL of diethyl phthalate add a solution of tetra-*n*-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 100 mL. To 6 mL of this solution add a solution of tetra-*n*-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 50 mL, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Cefetamet Pivoxil Hydrochloride: any peaks other than peaks of diethyl phthalate and the solvent are not observed.

**Diethyl terephthalate**  $C_6H_4(COOC_2H_5)_2$  White to pale brownish white, crystalline or mass.

*Melting point* <2.60>: 44 – 46°C

*Content*: not less than 99%. *Assay*—Dissolve 100 mg of diethyl terephthalate in 10 mL of methanol. Perform the test with 2  $\mu$ L of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content (\%)} = \frac{\text{peak area of diethyl terephthalate}}{\text{total of all peak areas}} \times 100$$

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass tube 4 mm in inside diameter and 2 m in length, packed with Shimalite W(AW, DMCS) coated with SE-30 in 10% (177 – 250  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 200°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of diethyl terephthalate is between 6 and 7 minutes.

Time span of measurement: About 5 times as long as the retention time of diethyl terephthalate beginning after the solvent peak.

**Difenidol hydrochloride**  $C_{21}H_{27}NO \cdot HCl$  [Same as the namesake monograph]

**Digitonin**  $C_{56}H_{92}O_{29}$  White to whitish crystals or crystalline powder.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : –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 20 mL of ethanol (95) by warming, and add ethanol (95) to make 50 mL. To 0.5 mL of this solution add 10 mL of a solution of cholesterol in ethanol (95) (1 in 5000), cool to 10°C, and allow to stand for 30 minutes while vigorous shaking occasionally: A precipitate is produced.

**Digoxin**  $C_{41}H_{64}O_{14}$  [Same as the namesake monograph]

**Dihydrocodeine phosphate for assay**  $C_{18}H_{23}NO_3 \cdot H_3PO_4$  [Same as the monograph Dihydrocodeine Phosphate. It contains not less than 99.0% of dihydrocodeine phosphate ( $C_{18}H_{23}NO_3 \cdot H_3PO_4$ ), calculated on the dried basis.]

**Dihydroergocristine mesilate for thin-layer chromatography**  $C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$  A pale yellowish white powder. Freely soluble in methanol, in ethanol (95) and in chloroform, sparingly soluble in water. Melting point: about 190°C (with decomposition).

*Purity* Related substances—Dissolve 6 mg of dihydroergocristine mesilate for thin-layer chromatography in exact 100 mL of a mixture of chloroform and methanol (9:1), and perform the test with 5  $\mu$ L of this solution as directed in the Purity (3) under Dihydroergotoxine Mesilate: any spot other than the principal spot at the *Rf* value around 0.4 does not appear.

**1-[(2*R*,5*S*)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl] thymine for thin-layer chromatography**  $C_{10}H_{12}N_2O_4$  Occurs as a white powder.

*Purity*—Dissolve 0.1 g of 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatog-

raphy in 100 mL of methanol and perform the test as directed in the Purity (2) under Zidovudine: spots other than the principal spot with an *Rf* value of about 0.23 are not observed.

**3,4-Dihydro-6-hydroxy-2(1*H*)-quinolinone**  $C_9H_9NO_2$  A white to light brown powder or granule. Melting point: about 240°C (with decomposition).

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3210  $cm^{-1}$ , 1649  $cm^{-1}$ , 1502  $cm^{-1}$ , 1252  $cm^{-1}$  and 816  $cm^{-1}$ .

**2,4-Dihydroxybenzoic acid**  $C_7H_6O_4$  White to pale brown powder.

*Purity* Clarity of solution—Dissolve 1.0 g of 2,4-dihydroxybenzoic acid in 20 mL of ethanol (95): the solution is clear.

*Content*: not less than 95%. *Assay*—Weigh accurately about 1 g of 2,4-dihydroxybenzoic acid, dissolve in 50 mL of ethanol (95) and 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 15.41 \text{ mg of } C_7H_6O_4 \end{aligned}$$

**1,3-Dihydroxynaphthalene**  $C_{10}H_6(OH)_2$  Purple-brown, crystals or powder. Freely soluble in water and in ethanol (95).

*Melting point* <2.60>: about 125°C

**2,7-Dihydroxynaphthalene**  $C_{10}H_6(OH)_2$

*Purity*: not less than 97.0%.

**2,7-Dihydroxynaphthalene TS** Dissolve 0.10 g of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow to stand until the yellow color initially developed disappears. If the solution is blackened notably, prepare freshly.

**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* <2.45>  $n_D^{20}$ : 1.391 – 1.394

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.715 – 0.722

**Diltiazem hydrochloride**  $C_{22}H_{26}N_2O_4S \cdot HCl$  [Same as the namesake monograph]

**Dilute acetic acid** See acetic acid, dilute.

**Dilute bismuth subnitrate-potassium iodide TS for spray** Dissolve 10 g of L-tartaric acid in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

**Dilute bromophenol blue TS** See bromophenol blue TS, dilute.

**Dilute p-dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**Dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

**Diluted ethanol** See ethanol, diluted.

**Dilute ethanol** See ethanol, dilute.

**Dilute ferric ammonium sulfate TS** See ammonium iron

(III) sulfate TS, dilute.

**Dilute ferric chloride TS** See iron (III) 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 potassium hydroxide-ethanol TS** See potassium hydroxide-ethanol TS, dilute.

**Dilute sodium hydroxide TS** See sodium hydroxide TS, dilute.

**Dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS** To 5 mL of a solution of sodium pentacyanonitrosylferrate (III) dihydrate (3 in 50), 5 mL of a solution of potassium hexacyanoferrate (III) (13 in 200) and 2.5 mL of a solution of sodium hydroxide (1 in 10) add water to make 25 mL, and mix. Use after the color of the solution changes from dark red to light yellow. Prepare at the time of use.

**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.

**Dimedon**  $C_8H_{12}O_2$  White to pale yellow, crystalline powder.

*Melting point* <2.60>: 145 – 149°C

**Dimenhydrinate for assay**  $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$   
[Same as the monograph Dimenhydrinate. When dried, it contains not less than 53.8% and not more than 54.9% of diphenhydramine ( $C_{17}H_{21}NO$ ) and not less than 45.2% and not more than 46.1% of 8-chlorotheophylline ( $C_7H_7ClN_4O_2$ ).]

**Dimethoxymethane**  $C_3H_8O_2$  Colorless, clear and volatile liquid. Miscible with methanol, with ethanol (95) and with diethyl ether.

***N,N*-Dimethylacetamide**  $CH_3CON(CH_3)_2$  Clear and colorless liquid.

*Specific gravity* <2.56> *d*: 0.938 – 0.945 (Method 3).

*Boiling point* <2.57>: 163 – 165°C

*Water* <2.48>: not more than 0.2% (0.1 g, Coulometric titration).

*Purity*—Perform the test with 3  $\mu$ L of *N,N*-dimethylacetamide as directed under Gas Chromatography <2.02> 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 flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter

and 30 m in length, coated the inside surface 0.5  $\mu$ m in thickness with polyethylene glycol 20 M for gas chromatography.

Column temperature: The sample is injected at a constant temperature of about 70°C, keep this temperature for 1 minute, then raise to 200°C in 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.

Time span of measurement: About 2 times as long as the retention time of *N,N*-dimethylacetamide.

System suitability

Test for required detection: 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*-dimethylacetamide obtained from 3  $\mu$ L of this solution is equivalent to 40 to 60% of the full-scale.

System repeatability: When the test is repeated with 3  $\mu$ L 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%.

**Dimethylamine**  $(CH_3)_2NH$  Colorless, clear liquid, having amine-like, characteristic odor. It is miscible with water and with ethanol (99.5). It is alkaline.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.85 – 0.93

*Content*: 38.0 – 45.0%. Assay—Weigh accurately about 1 g of dimethylamine, transfer to a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> 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.

Each mL of 0.5 mol/L sulfuric acid VS  
= 45.08 mg of  $(CH_3)_2NH$

**4-Dimethylaminoantipyrine**  $C_{13}H_{17}N_3O$  Colorless or white crystals, or a white crystalline powder.

*Purity* Related substances—Proceed the test with 5  $\mu$ L of a solution of 4-dimethylaminoantipyrine (1 in 2000) as directed in the Assay under Cefpiramide Sodium, determine each peak area in a range of about 2 times as long as the retention time of 4-dimethylaminoantipyrine after the solvent peak by the automatic integration method, and calculate the total amount of the peaks other than 4-dimethylaminoantipyrine by the area percentage method: not more than 1.0%.

**(Dimethylamino)azobenzenesulfonyl chloride**

$C_{14}H_{14}ClN_3O_2S$  Prepared for amino acid analysis or biochemistry.

***p*-Dimethylaminobenzaldehyde** See 4-dimethylaminobenzaldehyde.

**4-Dimethylaminobenzaldehyde**  $(CH_3)_2NC_6H_4CHO$   
[K 8496, *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 TS, dilute.

***p*-Dimethylaminobenzaldehyde-hydrochloric acid TS** See 4-dimethylaminobenzaldehyde-hydrochloric acid TS.

**4-Dimethylaminobenzaldehyde-hydrochloric acid TS**

Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 50 mL of hydrochloric acid while cooling, and add 50 mL of ethanol (95).

**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-dimethylaminobenzaldehyde-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 4-dimethylaminobenzaldehyde TS for spraying.

**4-Dimethylaminobenzaldehyde TS for spraying**

Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20 mL of dilute sulfuric acid. Prepare before use.

**p-Dimethylaminobenzylidene rhodanine** See 4-dimethylaminobenzylidene rhodanine.

**4-Dimethylaminobenzylidene rhodanine**  $C_{12}H_{12}N_2OS_2$  [K 8495, Special class]

**p-Dimethylaminobenzylidene rhodanine TS** See 4-dimethylaminobenzylidene rhodanine TS.

**4-Dimethylaminobenzylidene rhodanine TS** Dissolve 20 mg of 4-dimethylaminobenzylidene rhodanine 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) and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 140 – 142°C

*Purity* Clarity of solution—Dissolve 0.20 g of 4-dimethylaminocinnamaldehyde in 20 mL of ethanol (95); the solution is clear.

*Loss on drying* <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Nitrogen content* <1.08>: 7.8 – 8.1% (105°C, 2 hours, after drying).

**p-Dimethylaminocinnamaldehyde TS** See 4-dimethylaminocinnamaldehyde TS.

**4-Dimethylaminocinnamaldehyde TS** Before use, add 1 mL of acetic acid (100) to 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 2000).

**Dimethylaminophenol**  $(CH_3)_2NC_6H_4OH$  Dark purple, crystals or crystalline mass.

*Melting point* <2.60>: 85°C

**Dimethylaniline** See *N,N*-dimethylaniline.

***N,N*-Dimethylaniline**  $C_6H_5N(CH_3)_2$  Colorless or light yellow liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.955 – 0.960

*Distilling range* <2.57>: 192 – 195°C, not less than 95 vol%.

**Dimethylformamide** See *N,N*-dimethylformamide.

***N,N*-Dimethylformamide**  $HCON(CH_3)_2$  [K 8500, Special class]

***N,N*-Dimethylformamide for liquid chromatography**  $HCON(CH_3)_2$  [K 8500, *N,N*-Dimethylformamide, Special class] Read absorbance as directed under Ultraviolet-visible Spectrophotometry <2.24> (in a 1-cm cell, using water as the blank): the absorbance is not more than 0.60 at 270 nm, not more than 0.15 at 280 nm, and not more than 0.05 at 300 nm.

**Dimethylglyoxime**  $C_4H_8N_2O_2$  [K 8498, Special class]

**Dimethylglyoxime-thiosemicarbazide TS** Solution A: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL. Prepare before use. Solution B: Dissolve 0.1 g of thiosemicarbazide in 50 mL of water 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 100 mL.

**Dimethyl malonate**  $C_5H_8O_4$  Clear, colorless or pale yellow liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.152 – 1.162

*Water* <2.48>: not more than 0.3%.

*Residue on ignition* <2.44>: not more than 0.1%.

***N,N*-Dimethyl-*n*-octylamine**  $C_{10}H_{23}N$  Colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.424

***N,N*-Dimethyl-*p*-phenylenediammonium dichloride**  $H_2NC_6H_4N(CH_3)_2 \cdot 2HCl$  [K 8193, *N,N*-Dimethyl-*p*-phenylenediammonium dichloride, Special class]

***N,N*-Dimethyl-*p*-phenylenediammonium hydrochloride** See *N,N*-dimethyl-*p*-phenylenediamine dichloride.

**Dimethyl phthalate**  $C_{16}H_{22}O_4$  Colorless, clear liquid, having a slight aroma.

*Refractive index* <2.45>  $n_D^{20}$ : 1.491 – 1.493

*Purity*—To 6.0 mL of a solution of Dimethyl phthalate in isoctane (1 in 100) add a solution of *n*-amyl alcohol in hexane (3 in 1000) to make 50 mL, and perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay under Ergocalciferol or Cholecalciferol: any peak other than the principal peak does not appear.

**Dimethylsulfoxide**  $(CH_3)_2SO$  [K 9702, Special class]

**Dimethylsulfoxide for ultraviolet-visible spectrophotometry**  $(CH_3)_2SO$  Colorless crystals or clear colorless liquid, having a characteristic odor. It is highly hygroscopic.

*Congearing point* <2.42>: not less than 18.3°C.

*Purity*—Read absorbance of dimethylsulfoxide for ultra-

violet-visible spectrophotometry, immediately after saturating with nitrogen, using water as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: its value is not more than 0.20 at 270 nm, not more than 0.09 at 275 nm, not more than 0.06 at 280 nm, and not more than 0.015 at 300 nm. It exhibits no characteristic absorption between 260 nm and 350 nm.

*Water* <2.48>: not more than 0.1%.

**2,6-Dimethyl-4-(2-nitrosophenyl)3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography**

$C_{17}H_{16}N_2O_5$  Irradiate xenon light at 50,000 lx of illumination for 8 hours to a methanol solution of nifedipine (1 in 100), and evaporate the methanol on a water bath. Recrystallize the residue 4 times from 1-propanol, and dry in a desiccator (in vacuum, phosphorus pentoxide). Pale blue crystals. Very soluble in chloroform, freely soluble in acetone, and practically insoluble in water.

*Melting point* <2.60>: 93 – 95°C

*Content*: not less than 99.0%. *Assay*—Weigh accurately about 0.4 g of 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS  
= 32.83 mg of  $C_{17}H_{16}N_2O_5$

**3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide**  $C_{18}H_{16}BrN_5S$  Yellow crystals. Melting point: about 195°C (with decomposition).

**3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS** Dissolve 5 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in phosphate-buffered sodium chloride TS to make 1000 mL.

**Dimorpholamine for assay** [Same as the monograph Dimorpholamine. When dried, it contains not less than 99.0% of dimorpholamine ( $C_{20}H_{38}N_4O_4$ ).

*m*-Dinitrobenzene See 1,3-dinitrobenzene.

**1,2-Dinitrobenzene**  $C_6H_4(NO_2)_2$  Occurs as yellowish white to brownish yellow crystals or a crystalline powder.

*Identification*—Determine the infrared absorption spectrum of 1,2-dinitrobenzene as directed in the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3100\text{ cm}^{-1}$ ,  $1585\text{ cm}^{-1}$ ,  $1526\text{ cm}^{-1}$ ,  $1352\text{ cm}^{-1}$ , and  $793\text{ cm}^{-1}$ .

*Melting point* <2.60>: 116 – 119°C

**1,3-Dinitrobenzene**  $C_6H_4(NO_2)_2$  Light yellow to reddish-yellow crystals or crystalline powder.

*Melting point* <2.60>: 88 – 92°C.

Preserve in a light-resistant tight container.

*m*-Dinitrobenzene TS See 1,3-dinitrobenzene TS.

**1,3-Dinitrobenzene TS** Dissolve 1 g of 1,3-dinitrobenzene in 100 mL of ethanol (95). Prepare before use.

*m*-Dinitrobenzene TS, alkaline See 1,3-dinitrobenzene TS, alkaline.

**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).

**2,4-Dinitrochlorobenzene** See 1-chloro-2, 4-dinitrobenzene.

**2,4-Dinitrofluorobenzene** See 1-fluoro-2, 4-dinitrobenzene.

**2,4-Dinitrophenol**  $C_6H_3OH(NO_2)_2$  Yellow crystals or crystalline powder.

*Melting point* <2.60>: 110 – 114°C

**2,4-Dinitrophenol TS** Dissolve 0.5 g of 2,4-dinitrophenol in 100 mL of ethanol (95).

**2,4-Dinitrophenylhydrazine**  $(NO_2)_2C_6H_3NHNH_2$   
[K 8480, Special class]

**2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS** Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100 mL 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 10 mL of water, then add a mixture of 1 volume of aldehyde-free ethanol and 3 volumes of water to make 100 mL, 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* <2.56>  $d_{20}^{20}$ : 0.967 – 0.987

*Acid value* <1.13>: not more than 2.

**Dioxane** See 1,4-dioxane.

**1,4-Dioxane**  $C_4H_8O_2$  [K 8461, Special class]

**Diphenhydramine**  $C_{17}H_{21}NO$  [Same as the namesake monograph]

**Diphenhydramine tannate** [Same as the namesake monograph]

**Diphenyl**  $C_{12}H_{10}$  White crystals or crystalline powder, having a characteristic odor. Freely soluble in acetone and in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

*Melting point* <2.60>: 68 – 72°C

*Purity*—Dissolve 0.1 g of diphenyl in 5 mL of acetone and use this solution as the sample solution. Perform the test with 2  $\mu$ L of this solution as directed under Gas Chromatography <2.02> 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 shows the purity of 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 150 to 180  $\mu$ m mesh siliceous earth for gas chromatography coated with 10% of

polyethylene glycol 20 M for thin-layer 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 the solution prepared by adding acetone to 1.0 mL of the sample 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 beginning after the solvent peak.

**Diphenylamine** (C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>NH [K 8487, Special class]

**Diphenylamine-acetic acid 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 (100) TS** See diphenylamine-acetic acid TS.

**Diphenylamine TS** Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

**9,10-Diphenylanthracene** C<sub>26</sub>H<sub>18</sub> Yellow crystalline powder. Soluble in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: about 248°C

**1,4-Diphenylbenzene** C<sub>18</sub>H<sub>14</sub> White scaly crystals, having a slight aromatic odor. It is freely soluble in ethanol (99.5), and slightly soluble in water.

*Identification*—Determine the infrared absorption spectrum of 1,4-diphenylbenzene as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3050 cm<sup>-1</sup>, 3020 cm<sup>-1</sup>, 1585 cm<sup>-1</sup>, 1565 cm<sup>-1</sup>, 1476 cm<sup>-1</sup>, 1450 cm<sup>-1</sup>, 995 cm<sup>-1</sup>, 834 cm<sup>-1</sup>, 740 cm<sup>-1</sup> and 680 cm<sup>-1</sup>.

**Diphenylcarbazine** See 1,5-diphenylcarbonohydrazide.

**Diphenylcarbazine TS** See 1,5-diphenylcarbonohydrazide TS.

**Diphenylcarbazone** C<sub>6</sub>H<sub>5</sub>N<sub>2</sub>CON<sub>2</sub>H<sub>2</sub>C<sub>6</sub>H<sub>5</sub> A yellowish-red crystalline powder.

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1708 cm<sup>-1</sup>, 1602 cm<sup>-1</sup>, 1497 cm<sup>-1</sup>, 1124 cm<sup>-1</sup>, 986 cm<sup>-1</sup>, 748 cm<sup>-1</sup> and 692 cm<sup>-1</sup>.

Preserve in a light-resistant tight container.

**Diphenylcarbazone TS** Dissolve 1 g of diphenylcarbazone in ethanol (95) to make 1000 mL.

**1,5-Diphenylcarbonohydrazide** C<sub>13</sub>H<sub>14</sub>N<sub>4</sub>O [K 8488, Special class]

**1,5-Diphenylcarbonohydrazide TS** Dissolve 0.2 g of 1,5-diphenylcarbonohydrazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9:1).

**5% Diphenyl-95% dimethylpolysiloxane for gas chromatography** Prepared for gas chromatography.

**Diphenyl ether** C<sub>12</sub>H<sub>10</sub>O Colorless crystals, having a geranium-like aroma. Dissolves in alcohol (95) and in diethyl

ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.072 – 1.075

*Boiling point* <2.57>: 254 – 259°C

*Melting point* <2.60>: 28°C

**Diphenyl imidazole** C<sub>15</sub>H<sub>12</sub>N<sub>2</sub> White crystals or crystalline powder, freely soluble in acetic acid (100), and sparingly soluble in methanol.

*Melting point* <2.60>: 234 – 238°C

*Loss on drying* <2.41>: not more than 0.5% (0.5 g, 105°C, 3 hours).

*Content*: not less than 99.0%. Assay—Dissolve about 0.3 g of diphenyl imidazole, previously dried and weighed accurately, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS).

Each mL of 0.1 mol/L perchloric acid VS  
= 22.03 mg of C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>

**Diphenyl phthalate** C<sub>6</sub>H<sub>4</sub>(COOC<sub>6</sub>H<sub>5</sub>)<sub>2</sub> White crystalline powder.

*Melting point* <2.60>: 71 – 76°C

*Purity* Related substances—Dissolve 60 mg of diphenyl phthalate in 50 mL of chloroform and use this solution as the sample solution. Proceed with 10 µL of the sample 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 sample 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.

**1,1-Diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography** C<sub>21</sub>H<sub>25</sub>N.HCl To 1 g of diphenidole hydrochloride add 30 mL of 1 mol/L hydrochloric acid TS, and heat under a reflux condenser for 1 hour. After cooling, extract twice with 30 mL-portions of chloroform, combine the chloroform extracts, wash twice with 10 mL portions of water, and evaporate chloroform under reduced pressure. Recrystallize the residue from a mixture of diethyl ether and ethanol (95) (3:1), and dry in a desiccator (in vacuum, silica gel) for 2 hours. White crystals or crystalline powder.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (250 nm): 386 – 446 (10 mg, water, 1000 mL).

*Melting point* <2.60>: 176 – 180°C

*Content*: not less than 99.0%. Assay—Dissolve about 0.2 g of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography, previously weighed accurately, in 20 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS  
= 16.39 mg of C<sub>21</sub>H<sub>25</sub>N.HCl

**Dipicolinic acid** C<sub>7</sub>H<sub>5</sub>NO<sub>4</sub> White powder.

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2630 cm<sup>-1</sup>, 1701 cm<sup>-1</sup>, 1576 cm<sup>-1</sup>, 1416 cm<sup>-1</sup>, 1300 cm<sup>-1</sup> and 1267 cm<sup>-1</sup>.

**Purity** Clarity and color of solution—Dissolve by warming 0.5 g in 20 mL of ethanol (99.5), and cool: a clear, colorless liquid.

**Content:** Not less than 98.0%. **Assay**—Weigh accurately about 0.1 g, add 25 mL of ethanol (99.5), dissolve by warming, cool, then titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 8.356 mg of  $C_7H_5O_4N$

**Dipotassium hydrogen phosphate**  $K_2HPO_4$  [K 9017, Special class]

**Dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3** Mix 100 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and 38 mL 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.

**Diprophylline**  $C_{10}H_{14}N_4O_4$  A white, powder or grain. Freely soluble in water, and slightly soluble in ethanol (95).

**Identification**—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3460  $cm^{-1}$ , 3330  $cm^{-1}$ , 1651  $cm^{-1}$ , 1242  $cm^{-1}$ , 1059  $cm^{-1}$  and 1035  $cm^{-1}$ .

$\alpha, \alpha'$ -Dipyridyl See 2,2'-bipyridyl.

**Disodium chlomotropate dihydrate**

$C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$  [K 8316, Special class] Preserve in light-resistant containers.

**Disodium dihydrogen ethylenediamine tetraacetate dihydrate**  $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$  [K 8107, Special class]

**0.1 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS** Dissolve 37.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

**0.04 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS** Dissolve 14.890 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

**0.4 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5** Dissolve 148.9 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in about 800 mL of water, adjust to pH 8.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**Disodium ethylenediaminetetraacetate** See disodium dihydrogen ethylenediamine tetraacetate dihydrate.

**Disodium ethylenediaminetetraacetate copper** See copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

**0.1 mol/L Disodium ethylenediaminetetraacetate TS** See 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

**Disodium hydrogen phosphate, anhydrous**  $Na_2HPO_4$   
[K 9020, Special class]

**Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0** Dissolve 35.8 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. To this solution add a solution of citric acid monohydrate (21 in 1000) to adjust the pH to 3.0.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 4.5** Dissolve 21.02 g of citric acid monohydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 35.82 g of disodium hydrogen phosphate 12-water in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 5.0** Dissolve 7.1 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and adjust to pH 5.0 with a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 5.4** Dissolve 1.05 g of citric acid monohydrate and 2.92 g of disodium hydrogen phosphate dodecahydrate in 200 mL of water, and adjust the pH with phosphoric acid or sodium hydroxide TS, if necessary.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0** Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate 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).

**0.05 mol/L Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0** To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust pH 6.0.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 7.2** Dissolve 7.1 g of disodium hydrogen phosphate in water to make 1000 mL. Adjust this solution to pH 7.2 with a solution prepared by dissolving 5.3 g of citric acid monohydrate in water to make 1000 mL.

**Disodium hydrogen phosphate-citric acid buffer solution, pH 7.5** Dissolve 5.25 g of citric acid monohydrate in water to make 1000 mL. Add this solution to 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS to adjust the pH to 7.5.

**Disodium hydrogen phosphate-citric acid buffer solution for penicillium origin  $\beta$ -galactosidase, pH 4.5** Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL (volume ratio: about 44:56).

**Disodium hydrogen phosphate dodecahydrate**  
 $Na_2HPO_4 \cdot 12H_2O$  [K 9019, Special class]

**Disodium hydrogen phosphate for pH determination**  
 $Na_2HPO_4$  [K 9020, for pH determination]

**Disodium hydrogen phosphate TS** Dissolve 12 g of disodium hydrogen phosphate dodecahydrate in water to make 100 mL (0.3 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.

**Disodium 1-nitroso-2-naphthol-3,6-disulfonate**  
 $C_{10}H_5NNa_2O_8S_2$  Yellow crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3400\text{ cm}^{-1}$ ,  $1639\text{ cm}^{-1}$ ,  $1451\text{ cm}^{-1}$ ,  $1270\text{ cm}^{-1}$ ,  $1231\text{ cm}^{-1}$ ,  $1173\text{ cm}^{-1}$ ,  $1049\text{ cm}^{-1}$ ,  $848\text{ cm}^{-1}$  and  $662\text{ cm}^{-1}$ .

Preserve in a light-resistant tight container.

**Dissolved acetylene**  $C_2H_2$  [K 1902]

**Distigmine bromide for assay**  $C_{22}H_{32}Br_2N_4O_4$  [Same as the monograph Distigmine Bromide. It contains not less than 99.0% of distigmine bromide ( $C_{22}H_{32}Br_2N_4O_4$ ), calculated on the anhydrous basis.]

**Distilled water for injection** [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers. Prepared by distillation. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

**2,6-Di-*tert*-butylcresol**  $[(CH_3)_3C]_2C_6H_2(CH_3)OH$  A white, crystalline powder. Freely soluble in ethanol (95).  
*Melting point* <2.60>: 69 – 71°C  
*Residue on ignition* <2.44>: not more than 0.05%.

**2,6-Di-*tert*-butylcresol TS** Dissolve 0.1 g of 2,6-di-*tert*-butylcresol in ethanol (95) to make 10 mL.

**2,6-Di-*tert*-butyl-*p*-cresol** See 2,6-di-*tert*-butylcresol.

**2,6-Di-*tert*-butyl-*p*-cresol TS** See 2,6-di-*tert*-butylcresol TS.

**1,3-Di (4-pyridyl) propane**  $C_{13}H_{14}N_2$  A pale yellow powder.  
*Melting point* <2.60>: 61 – 62°C  
*Water* <2.48>: less than 0.1%.

**1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-L-diproline**  
 $C_{18}H_{28}N_2O_6S_2$  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-diproline according to potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $2960\text{ cm}^{-1}$ ,  $1750\text{ cm}^{-1}$ ,  $1720\text{ cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1480\text{ cm}^{-1}$ ,  $1450\text{ cm}^{-1}$  and  $1185\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 0.10 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline in exactly 10 mL of methanol. Perform the test with this solution as directed in the Purity (3) under Captopril: any spot other than the principal spot at the *Rf* value of about 0.2 does not appear.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.3 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline, dissolve in 20 mL of methanol, add 50 mL of

water, and titrate <2.50> 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 determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
 = 21.63 mg of  $C_{18}H_{28}N_2O_6S_2$

**Dithiodiglycolic acid**  $C_4H_6O_4S_2$  Prepared for amino acid analysis or biochemistry.

**Dithiodipropionic acid**  $C_6H_{10}O_4S_2$  Prepared for amino acid analysis or biochemistry.

**Dithiothreitol**  $C_4H_{10}O_2S_2$  Crystals.  
*Melting point* <2.60>: about 42°C

**Dithizone**  $C_6H_5NHNHCSN:NC_6H_5$  [K 8490, 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 100 mL. Prepare before use.

**Dopamine hydrochloride for assay**  $C_8H_{11}NO_2.HCl$  [Same as the monograph Dopamine hydrochloride. When dried, it contains not less than 99.0% of dopamine hydrochloride ( $C_8H_{11}NO_2.HCl$ ).]

**Doxifluridine**  $C_9H_{11}FN_2O_5$  [Same as the namesake monograph]

**Doxifluridine for assay**  $C_9H_{11}FN_2O_5$  [Same as the monograph Doxifluridine. When dried, it contains not less than 99.5% of doxifluridine ( $C_9H_{11}FN_2O_5$ ).]

**Dragendorff's TS** Dissolve 0.85 g of bismuth subnitrate in 10 mL of acetic acid (100) and 40 mL of water with vigorous shaking (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). Immediately before use, mix equal volumes of solution A, solution B and acetic acid (100). Store solution A and solution B in light-resistant containers.

**Dragendorff's TS for spraying** Add 20 mL of diluted acetic acid (31) (1 in 5) to 4 mL of a mixture of equal volumes of solution A and solution B of Dragendorff's TS. Prepare before use.

**Dried human normal plasma powder** Freeze-dried normal plasma obtained from healthy human.

**Dried sodium carbonate**  $Na_2CO_3$  [Same as the name-sake monograph]

**Droxidopa for assay**  $C_9H_{11}NO_5$  [Same as the monograph Droxidopa. When dried, it contains not less than 99.5% of droxidopa ( $C_9H_{11}NO_5$ ).]

**Dydrogesterone for assay**  $C_{21}H_{28}O_2$  [Same as the monograph Dydrogesterone. When dried, it contains not less than 99.0% of  $C_{21}H_{28}O_2$ ]

**Ebastine for assay**  $C_{32}H_{39}NO_2$  [Same as the monograph

Ebastine. When dried, it contains not less than 99.5% of ebastine ( $C_{32}H_{39}NO_2$ ).]

**Ecabet sodium hydrate for assay**  $C_{20}H_{27}NaO_5S \cdot 5H_2O$  [Same as the monograph Ecabet Sodium Hydrate. It contains not less than 99.5% of ecabet sodium ( $C_{20}H_{27}NaO_5S$ ), calculated on the anhydrous basis.]

**E. coli protein** Process E. coli cells (*E. coli* N4830/pTB281) retaining a plasmid deficient in the celmoleukin gene according to the celmoleukin purification process in the following order; (i) extraction, (ii) butylated vinyl polymer hydrophobic chromatography, (iii) carboxymethylated vinyl polymer ion-exchange column chromatography, and (iv) sulfopropyl-polymer ion-exchange chromatography, and during process (iv) collect the fractions corresponding to the celmoleukin elution position. Dialyze the fractions obtained in (iv) against 0.01 mol/L acetate buffer solution, pH 5.0, and take the dialysis solution as E.coli protein.

*Description*—Clear and colorless solution.

*Identification*—When the absorption spectrum is determined using UV absorption photometry <2.24>, an absorption maximum is observed in the region of 278 nm.

*Protein content*: When determining the protein content using the Assay (1) Total protein content under Celmoleukin (Genetical Recombination), the protein content per mL is 0.1 to 0.5 mg.

**E. coli protein stock solution** A solution obtained by culturing a bacteria that contains a plasmid lacking the teceleukin gene but is otherwise exactly identical to the teceleukin-producing E. coli strain in every function except teceleukin production, and then purified using a purification technique that is more simple than that for teceleukin. Determine the amount of protein by Bradford method using bovine serum albumin as the standard substance. Store shielded from light at  $-70^\circ\text{C}$ .

#### Eleutheroside B for liquid chromatography

$C_{17}H_{24}O_9 \cdot xH_2O$  A white crystalline powder. Sparingly soluble in methanol, slightly soluble in water, and very slightly soluble in ethanol (99.5). Melting point:  $190-194^\circ\text{C}$ .

*Identification*—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

*Purity* Related substances—Dissolve 1.0 mg in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly  $10\ \mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of eleutheroside B is not larger than the peak area of eleutheroside B obtained with the standard solution.

*Operating conditions*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Identification under Eleutherococcus Senticosus Rhizome.

Time span of measurement: About 3 times as long as the

retention time of eleutheroside B beginning after the solvent peak.

*System suitability*

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of eleutheroside B obtained with  $10\ \mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with  $10\ \mu\text{L}$  of the standard solution.

*System performance*: Proceed as directed in the system suitability in the Identification under Eleutherococcus Senticosus Rhizome.

**EMB plate medium** Melt eosin methylene blue agar medium by heating, and cool to about  $50^\circ\text{C}$ . Transfer about 20 mL 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.

#### Emetine hydrochloride for assay

$C_{29}H_{40}N_2O_4 \cdot 2HCl \cdot xH_2O$  A white or light-yellow crystalline powder. Soluble in water.

*Melting point* <2.60>: about  $250^\circ\text{C}$  [with decomposition, after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide,  $50^\circ\text{C}$ ) for 5 hours].

*Absorbance* <2.24>  $E_{1\%}^{1\text{cm}}$  (283 nm): 116 – 127 (10 mg, diluted methanol (1 in 2), 400 mL) [after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide,  $50^\circ\text{C}$ ) for 5 hours].]

*Purity* Related substances—Dissolve 10 mg of emetine hydrochloride for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly  $10\ \mu\text{L}$  each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of peaks other than emetine from the sample solution is not larger than the peak of emetine from the standard solution (1).

*Operating conditions*

Proceed the operating conditions in the Assay under Ipecac except the detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the sensitivity so that the peak area of emetine obtained from  $10\ \mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method, and the peak height of emetine obtained from  $10\ \mu\text{L}$  of the standard solution (1) is about 20 % of the full scale.

Time span of measurement: About 3 times as long as the retention time of emetine beginning after the solvent peak.

**Emetine for component determination** See emetine for assay.

**Emorfazone for assay**  $C_{11}H_{17}N_3O_3$  [Same as the monograph Emorfazone. When dried, it contains not less than 99.0% of emorfazone ( $C_{11}H_{17}N_3O_3$ ).]

**Enalapril maleate**  $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$  [Same as the namesake monograph]

**Endo's medium** Melt 1000 mL of the ordinary agar me-

dium by heating in a water bath, and adjust the pH to between 7.5 and 7.8. Add 10 g of lactose monohydrate previously dissolved in a small quantity of water, mix thoroughly, and add 1 mL of fuchsin-ethanol (95) TS. After cooling to about 50°C, add dropwise a freshly prepared solution of sodium bisulfite (1 in 10) until a light red color develops owing to reducing fuchsin, requiring about 10 to 15 mL of a solution of sodium sulfite heptahydrate (1 in 10). Dispense the mixture, and sterilize fractionally on each of three successive days for 15 minutes at 100°C.

**Endo's plate medium** Melt Endo's medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dishes with the cover slightly opened in the incubator to evaporate the inner vapor and water on the surface of the agar.

**Enflurane**  $C_3H_2ClF_5O$  [Same as the namesake monograph]

**Enzyme TS** The supernatant liquid is obtained as follows: To 0.3 g of an enzyme preparation potent in amyolytic and phospholytic 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 Y**  $C_{20}H_6Br_4Na_2O_5$  Red, masses or powder.

**Identification**—To 10 mL of a solution (1 in 1000) add 1 drop of hydrochloric acid: yellow-red precipitates appear.

**Eosin methylene blue agar medium** Dissolve by boiling 10 g of casein peptone, 2 g of dipotassium hydrogenphosphate and 25 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), 13 mL 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.

**Ephedrine hydrochloride**  $C_{10}H_{15}NO.HCl$  [Same as the namesake monograph]

**Ephedrine hydrochloride for assay** See ephedrine hydrochloride.

**6-Epidoxycycline hydrochloride**  $C_{22}H_{24}N_2O_8.HCl$  Yellow to dark yellow, crystals or crystalline powder.

**Purity** Related substances—Dissolve 20 mg of 6-epidoxycycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of the peaks other than 6-epidoxycycline is not more than 10%.

**4-Epioxytetracycline**  $C_{22}H_{24}N_2O_9$  Green-brown to brown powder.

**Purity** Related substances—Dissolve 20 mg of 4-epioxytetracycline in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity

(2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than 4-epioxytetracycline is not more than 10%.

**Eriochrome black T**  $C_{20}H_{12}N_3NaO_7S$  [K 8736, Special class]

**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 hydroxylammonium chloride in methanol to make 50 mL. Use within 1 week. Preserve in light-resistant containers.

**Erythromycin B**  $C_{37}H_{67}NO_{12}$  White to light yellowish white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin B in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100  $\mu$ L each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin B from the sample solution is not more than the peak area of erythromycin B from the standard solution.

**Erythromycin C**  $C_{36}H_{65}NO_{13}$  White to light yellowish white powder.

**Purity** Related substances—Dissolve 10 mg of erythromycin C in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100  $\mu$ L each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin C from the sample solution is not more than the peak area of erythromycin C from the standard solution.

**Essential oil** Same as the essential oil under the monograph.

**Etacrynic acid for assay** [Same as the monograph Etacrynic acid. When dried, it contains not less than 99.0% of etacrynic acid ( $C_{13}H_{12}Cl_2O_4$ ).]

**Ethanol** See ethanol (95).

**Ethanol, aldehyde-free** Transfer 1000 mL of ethanol (95) to a glass-stoppered bottle, add the 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 so-

lution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the supernatant liquid, and distil the ethanol.

**Ethanol, dehydrated** 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 diluting ethanol (99.5).

**Ethanol for alcohol number determination** See Alcohol Number Determination <1.01>.

**Ethanol for disinfection** [Same as the namesake monograph]

**Ethanol for gas chromatography** Use ethanol prepared by distilling ethanol (99.5) with iron (II) sulfate heptahydrate. Preserve in containers, in which the air has been displaced with nitrogen, in a dark, cold place.

**Ethanol-free chloroform** See chloroform, ethanol-free.

**Ethanol-isotonic sodium chloride solution** To 1 volume of ethanol (95) add 19 volumes of isotonic sodium chloride solution.

**Ethanol, methanol-free** See ethanol (95), methanol-free.

**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)**  $C_2H_5OH$  [K 8102, Special class]

**Ethanol (95), methanol-free** Perform the test for methanol, by using this methanol-free ethanol (95) in place of the standard solution, as directed in Methanol Test <1.12>: it is practically colorless.

**Ethanol (99.5)**  $C_2H_5OH$  [K 8101, Special class]

**Ethenzamide**  $C_9H_{11}NO_2$  [Same as the namesake monograph].

**Ether** See diethyl ether.

**Ether, anesthetic**  $C_2H_5OC_2H_5$  [Same as the namesake monograph]

**Ether, dehydrated** See diethyl ether, dehydrated.

**Ether for purity of crude drug** See diethyl ether for purity of crude drug.

**Ethinylestradiol**  $C_{20}H_{24}O_2$  [Same as the namesake monograph]

**3-Ethoxy-4-hydroxybenzaldehyde**  $C_9H_{10}O_3$  White to pale yellowish white crystalline. Freely soluble in ethanol (95), and slightly soluble in water.

*Melting point* <2.60>: 76 – 78°C

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 0.3 g of 3-ethoxy-4-hydroxybenzaldehyde, previously dried in a desiccator (phosphorous (V) oxide) for 4 hours, dissolve in 50 mL of *N,N*-dimethylacetamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: thymol blue TS).

Each mL of 0.1 mol/L sodium methoxide VS  
= 16.62 mg of  $C_9H_{10}O_3$

**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* <2.60>: 62 – 68°C

*Purity*—Dissolve 0.5 g of 4-Ethoxyphenol in 5 mL of ethanol (95), and use this solution as the sample solution. Perform the test as directed under Gas Chromatography <2.02> 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 180- to 250- $\mu$ m siliceous earth for gas chromatography coated with methyl-silicone polymer 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  $\mu$ L of the sample 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 beginning after the solvent peak.

**Ethyl acetate**  $CH_3COOC_2H_5$  [K 8361, Special class]

**Ethyl aminobenzoate**  $C_9H_{11}NO_2$  [Same as the namesake monograph]

**Ethylbenzene**  $C_6H_5C_2H_5$  A colorless liquid. Freely soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.862 – 0.872

*Boiling point* <2.57>: about 135°C

**Ethyl benzoate**  $C_6H_5COOC_2H_5$  Clear, colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.502 – 1.507

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.045 – 1.053

**Ethyl n-caprylate**  $C_{10}H_{20}O_2$  Clear and colorless to almost colorless liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.864 – 0.871

*Purity* Related substances—Dissolve 0.1 g of ethyl *n*-caprylate in 10 mL 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  $\mu$ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> 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  $\mu$ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of ethyl *n*-caprylate from 5  $\mu$ L 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.

**Ethyl carbamate**  $\text{H}_2\text{NCOOC}_2\text{H}_5$  White crystals or powder.

*Melting point* <2.60>: 48 – 50°C

*Purity* Clarity of solution—Dissolve 5 g in 20 mL of water: the solution is clear.

**Ethyl cyanoacetate**  $\text{NCCH}_2\text{COOC}_2\text{H}_5$  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 1 mL 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**  $\text{C}_2\text{H}_8\text{N}_2$  [Same as the namesake monograph]

**Ethylenediamine TS** Dissolve 70 g of ethylenediamine in 30 g of water.

**Ethylene glycol**  $\text{HOCH}_2\text{CH}_2\text{OH}$  [K 8105, Special class]

**Ethylene glycol for Karl Fischer method** Distil ethylene glycol, and collect the fraction distilling between 195°C and 198°C. The water content is not more than 1.0 mg per mL.

**2-Ethylhexyl parahydroxybenzoate**  $\text{C}_{15}\text{H}_{22}\text{O}_3$  Pale yellow, clear viscous liquid. Miscible with methanol (99.5). Practically insoluble in water.

*Content*: not less than 98.0%. *Assay*—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS  
= 250.3 mg of  $\text{C}_{15}\text{H}_{22}\text{O}_3$

**Ethyl iodide** See iodoethane.

**N-Ethylmaleimide**  $\text{C}_6\text{H}_7\text{NO}_2$  White crystals, having a pungent, characteristic odor. Freely soluble in ethanol (95), and slightly soluble in water.

*Melting point* <2.60>: 43 – 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 <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 12.51 mg of  $\text{C}_6\text{H}_7\text{NO}_2$

**Ethyl parahydroxybenzoate**  $\text{HOC}_6\text{H}_4\text{COOC}_2\text{H}_5$  [Same as the namesake monograph]

**2-Ethyl-2-phenylmalondiamide**  $\text{C}_{11}\text{H}_{14}\text{O}_2\text{N}_2$  White, odorless crystals. Soluble in ethanol (95), and very slightly soluble in water. Melting point: about 120°C (with decomposition).

*Purity* Related substances—To 5.0 mg of 2-ethyl-2-phenylmalondiamide add 4 mL of pyridine and 1 mL of bis-trimethylsilylacetamide, shake thoroughly, and heat at 100°C for 5 minutes. After cooling, add pyridine to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the conditions in the Purity (3) under Primidone: any peak other than the peaks of 2-ethyl-2-phenylmalondiamide and the solvent does not appear. Adjust the detection sensitivity so that the peak height of 2-ethyl-2-phenylmalondiamide obtained from 2  $\mu$ L of the sample solution is about 80% of the full scale, and the time span of measurement is about twice as long as the retention time of 2-ethyl-2-phenylmalondiamide beginning after the solvent peak.

**Ethyl propionate**  $\text{CH}_3\text{CH}_2\text{COOC}_2\text{H}_5$  Colorless, clear liquid.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.890 – 0.892

**Etidronate disodium for assay**  $\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$  [Same as the monograph Etidronate Disodium. When dried, it contains not less than 99.0% of  $\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$ ]

**Etilefrine hydrochloride**  $\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$  [Same as the namesake monograph]

**Etilefrine hydrochloride for assay**  $\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$  [Same as the monograph Etilefrine Hydrochloride. When dried, it contains not less than 99.0% of etilefrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$ ).]

**Etizolam for assay**  $\text{C}_{17}\text{H}_{15}\text{ClN}_4\text{S}$  [Same as the monograph Etizolam. When dried, it contains not less than 99.0% of etizolam ( $\text{C}_{17}\text{H}_{15}\text{ClN}_4\text{S}$ ).]

**Factor Xa** It is prepared from lyophilization of Factor Xa which has been prepared from bovine plasma. White or pale yellow masses or powder.

*Purity* Clarity and color of solution—Dissolve 71  $\text{nk}_{\text{at}_{s-2222}}$  of it in 10 mL water; the solution is clear and colorless or pale yellow.

*Content*: not less than 75% and not more than 125% of the label.

**Factor Xa TS** Dissolve 71  $\text{nk}_{\text{at}_{s-2222}}$  of factor Xa in 10 mL of water.

**Famotidine for assay**  $\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$  [Same as the monograph Famotidine. When dried, it contains not less than 99.0% of famotidine ( $\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$ ), and when proceed as directed in the Purity (3), the total related substance is not more than 0.4%.]

**Fatty oil** Same as the fatty oil under the monograph.

**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 a glass-stoppered bottles in well-filled.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hy-

dioxide in water to make 500 mL. Preserve this solution in a polyethylene container.

Before use, mix equal volumes of both solutions.

**Fehling's TS for amyolytic 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 sodium hydroxide in water to make exactly 500 mL. 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** See ammonium iron (III) sulfate dodecahydrate.

**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-pyridine TS, anhydrous** See iron (III) chloride-pyridin TS, anhydrous.

**Ferric chloride TS** See iron (III) chloride TS.

**Ferric chloride TS, acidic** See iron (III) chloride TS, acidic.

**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 TS.

**Ferric salicylate TS** See Iron salicylate TS

**Ferric sulfate** See iron (III) sulfate *n*-hydrate.

**Ferric sulfate TS** See iron (III) sulfate TS.

**Ferrous ammonium sulfate** See ammonium iron (II) sulfate hexahydrate.

**Ferrous sulfate** See iron (II) sulfate heptahydrate.

**Ferrous sulfate TS** See iron (II) sulfate TS.

**Ferrous sulfide** See iron (II) sulfide.

**Ferrous tartrate TS** See iron (II) tartrate TS.

**Ferrous thiocyanate TS** See iron (II) thiocyanate TS.

**Ferrous trisodium pentacyanoamine TS** See iron (II) trisodium pentacyanoamine TS.

**(E)-Ferulic acid**  $C_{10}H_{10}O_4$  White to light yellow, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 173 – 176°C.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, between 231 nm and 235 nm, and between 318 nm and 322 nm.

**Purity** Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2  $\mu$ L of this solution as directed in the Identification (11) under Hochuekkito Extract: no spot other than the principle spot of around Rf 0.6 appears.

**Fetal calf serum** Serum obtained from fetal calves. Interleukin-2 dependent cell growth suppression substance is removed by heat at 56°C for 30 min before use.

**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 1 mL of isotonic sodium chloride solution to 10 mg of fibrinogen. It, when warmed to 37°C, dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin.

**1st Fluid for disintegration test** See 1st fluid for dissolution test.

**1st Fluid for dissolution test** Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and water to make 1000 mL. It is clear and colorless, and has a pH of about 1.2.

**Fixed oil** Same as the vegetable oils under the monograph.

**Flecainide acetate**  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  [Same as the namesake monograph]

**Flecainide acetate for assay**  $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$  [Same as the monograph Flecainide Acetate. When dried, it contains not less than 99.0% of flecainide acetate ( $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$ ). Additionally, when perform the test as directed in the Purity (3), the sample solution does not show the spot corresponding to the spot obtained from the standard solution, and when perform the test as directed in the Purity (4), the total area of the peaks other than flecainide is not larger than the peak area of flecainide from the standard solution.]

**9-Fluorenylmethyl chloroformate**  $C_{15}H_{11}ClO_2$  White crystals or crystalline powder.

**Melting point** <2.60>: 60 – 63°C

**Flopropione**  $C_9H_{10}O_4$  [Same as the namesake monograph]

**Flopropione for assay**  $C_9H_{10}O_4$  [Same as the monograph Flopropione. It contains not less than 99.0% of flopropione ( $C_9H_{10}O_4$ : 182.17), calculated on the dehydrated basis.]

**Fluid thioglycolate medium** See the Sterility Test <4.06>.

**Fluocinolone acetonide**  $C_{24}H_{30}F_2O_6$  [Same as the name-sake monograph]

**9-Fluorenylmethyl chloroformate**  $C_{15}H_{11}ClO_2$   
Prepared for amino acid analysis or biochemistry.

**Fluorescein**  $C_{20}H_{12}O_5$  An yellowish red powder.

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $1597\text{ cm}^{-1}$ ,  $1466\text{ cm}^{-1}$ ,  $1389\text{ cm}^{-1}$ ,  $1317\text{ cm}^{-1}$ ,  $1264\text{ cm}^{-1}$ ,  $1247\text{ cm}^{-1}$ ,  $1213\text{ cm}^{-1}$ ,  $1114\text{ cm}^{-1}$  and  $849\text{ cm}^{-1}$ .

**Fluorescein sodium**  $C_{20}H_{10}Na_2O_5$  [Same as the name-sake monograph].

**Fluorescein sodium TS** Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

**4-Fluorobenzoic acid**  $C_7H_5FO_2$  White, crystals or crystalline powder.

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $1684\text{ cm}^{-1}$ ,  $1606\text{ cm}^{-1}$  and  $1231\text{ cm}^{-1}$ .

*Melting point* <2.60>:  $182 - 188^\circ\text{C}$

**1-Fluoro-2,4-dinitrobenzene**  $C_6H_3(NO_2)_2F$  Light yellow liquid or crystalline masses. Melting point: about  $25^\circ\text{C}$ .

*Identification*—Determine the infrared absorption spectrum as directed in the liquid film method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3110\text{ cm}^{-1}$ ,  $1617\text{ cm}^{-1}$ ,  $1538\text{ cm}^{-1}$ ,  $1345\text{ cm}^{-1}$ ,  $1262\text{ cm}^{-1}$  and  $743\text{ cm}^{-1}$ .

Preserve in a light-resistant tight container.

**7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole**  $C_6H_2FN_3O_3$   
Prepared for amino acid analysis or biochemistry.

**Flurazepam for assay**  $C_{21}H_{23}ClFN_3O$  [Same as the monograph Flurazepam. When dried, it contains not less than 99.0% of flurazepam ( $C_{21}H_{23}ClFN_3O$ ).]

**Flutoprazepam for assay**  $C_{19}H_{16}ClFN_2O$  [Same as the monograph Flutoprazepam. When dried, it contains not less than 99.5% of flutoprazepam ( $C_{19}H_{16}ClFN_2O$ ).]

**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 (VI) 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 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser, and cool. Add water to make 200 mL, and filter through a glass filter. Store it free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

**Folin's TS, dilute** Titrate <2.50> Folin's TS with 0.1 mol/L sodium hydroxide VS (indicator: phenolphthalein

TS), and determine the acid concentration. Prepare by adding water to Folin's TS so the acid concentration is 1 mol/L.

**Formaldehyde solution** HCHO [K 8872, Special class]

**Formaldehyde solution-sulfuric acid TS** Add 1 drop of formaldehyde solution to 1 mL of sulfuric acid. Prepare before use.

**Formaldehyde solution TS** To 0.5 mL of formaldehyde solution add water to make 100 mL.

**Formaldehyde TS, dilute** See Test Methods for Plastic Containers <7.02>.

**Formalin** See formaldehyde solution.

**Formalin TS** See formaldehyde solution TS.

**Formalin-sulfuric acid TS** See formaldehyde solution-sulfuric acid TS.

**Formamide** HCONH<sub>2</sub> [K 8873, Special class]

**Formamide for Karl Fischer method** HCONH<sub>2</sub>  
[K 8873, Special class; water content per g of formamide for Karl Fischer method should be not more than 1 mg.]

**Formic acid** HCOOH [K 8264, Special class, specific gravity: not less than 1.21].

**2-Formylbenzoic acid** CHOC<sub>6</sub>H<sub>4</sub>COOH White crystals. Melting point:  $97 - 99^\circ\text{C}$

*Content*: not less than 99.0%. *Assay*—Weigh accurately about 0.3 g of 2-formylbenzoic acid, previously dried (in vacuum, phosphorus (V) oxide, 3 hours), dissolve in 50 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 15.01 mg of C<sub>8</sub>H<sub>6</sub>O<sub>3</sub>

**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 namesake monograph]

**Fuchsin** A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95).

*Loss on drying* <2.41>: 17.5 - 20.0% (1 g,  $105^\circ\text{C}$ , 4 hours).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**Fuchsin-ethanol TS** Dissolve 11 g of fuchsin in 100 mL of ethanol (95).

**Fuchsin-sulfurous acid TS** Dissolve 0.2 g of fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution prepared by dissolving 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid and water to make 200 mL, and allow to stand for at least 1 hour. Prepare before use.

**Fumaric acid for thin-layer chromatography**  $C_4H_4O_4$   
White, crystalline powder, odorless, and has a characteristic acid taste.

*Purity*—Perform the test as directed in the Identification (5) under Clemastine Fumarate: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.8 does not appear.

**Fuming nitric acid** See nitric acid, fuming.

**Fuming sulfuric acid** See sulfuric acid, fuming.

**Furfural**  $C_5H_4O_2$  A clear, colorless liquid.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.160 – 1.165

*Distilling range* <2.57>: 160 – 163°C, not less than 95 vol%.

**D-Galactosamine hydrochloride**  $C_6H_{13}NO_5 \cdot HCl$  White powder. Melting point: about 180°C (with decomposition).

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +90 – +97° (1 g, water, 100 mL, 100 mm).

**Galactose** See D-galactose.

**D-Galactose**  $C_6H_{12}O_6$  White crystals, granules or powder.

*Identification*—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390  $cm^{-1}$ , 3210  $cm^{-1}$ , 3140  $cm^{-1}$ , 1151  $cm^{-1}$ , 1068  $cm^{-1}$ , 956  $cm^{-1}$ , 836  $cm^{-1}$ , 765  $cm^{-1}$  and 660  $cm^{-1}$ .

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +79 – +82° (desiccator (silica gel), 2.5 g after drying for 18 hours, diluted ammonia solution (28) (1 in 300), 25 mL, 100 mm).

**Gallic acid** See gallic acid monohydrate.

**Gallic acid monohydrate**  $C_6H_2(OH)_3COOH \cdot H_2O$

White to pale yellowish white, crystals or powder.

*Melting point* <2.60>: about 260°C (with decomposition).

**Gelatin** [Same as the namesake monograph]

**Gelatin, acid-treated** [Same as the monograph 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 potassium dihydrogen phosphate, 15.6 g of sodium dihydrogen phosphate dihydrate 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) (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 dihydrate, 5.96 g of disodium hydrogen phosphate dodecahydrate and 5.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g of gelatin to this solution by heating, and after cooling add water to make 600 mL.

**Gelatin-phosphate buffer solution, pH 7.4** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 39.50 mL of 0.2 mol/L sodium hydroxide VS and 50 mL of water. Dissolve 0.2 g of gelatin to this solution by heating, then after cooling adjust to pH 7.4 with 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

**Gelatin-tris buffer solution** Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.22 g of sodium chloride in 700 mL of water. Separately, dissolve 10 g of acid-treated gelatin in 200 mL of water by warming. After cooling, 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.

**Geniposide for assay** Use geniposide for thin-layer chromatography meeting the following additional specifications.

*Absorbance* <2.24>  $E_{1\%}^{1\text{cm}}$  (240 nm): 249 – 269 [10 mg dried in a desiccator (reduced pressure of not exceeding 0.67 kPa, phosphorus (V) oxide) for 24 hours, diluted methanol (1 in 2), 500 mL].

*Purity* Related substances—Dissolve 5 mg of geniposide for assay in 50 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than geniposide from the sample solution is not larger than the peak area of geniposide from the standard solution (1). Operating conditions

Proceed as directed in the Assay under Gardenia Fruit except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of geniposide obtained from 10  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method and the peak height of geniposide obtained from 10  $\mu\text{L}$  of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of geniposide beginning after the solvent peak.

**Geniposide for component determination** See geniposide for assay.

**Geniposide for thin-layer chromatography**  $C_{17}H_{24}O_{10}$  White crystals or crystalline powder. Melting point: 159 – 163°C.

*Purity* Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20  $\mu\text{L}$  of this solution as directed in the Identification (2) under Gardenia Fruit: any spot other than the principal spot at the  $R_f$  value of about 0.3 does not appear.

**Gentamicin B**  $C_{19}H_{38}N_4O_{10}$  White to pale yellowish white powder. Very soluble in water, and practically insoluble in ethanol (95).

*Content*: not less than 80.0%. Assay—Dissolve a suitable amount of gentamicin B in 0.05 mol/L sulfuric acid TS to make the solution containing 0.1 mg of gentamicin B per mL, and use this solution as the sample solution. Perform the test with 5  $\mu\text{L}$  of the sample solution as directed under Liquid Chromatography <2.01> according to the following

conditions, and measure each peak area by the automatic integration method. Calculate the amount of gentamicin B by the area percentage method.

#### Operating conditions

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 3 times as long as the retention time of gentamicin B.

#### System suitability

Proceed the system suitability in the Assay under Isepamicin Sulfate.

**Gentiopicroside for thin-layer chromatography**  $C_{16}H_{20}O_9$   
A white powder. Freely soluble in water and in methanol, and practically insoluble in diethyl ether. Melting point: about 110°C (with decomposition).

**Purity** Related substances—Dissolve 10 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Gentian: the spots other than the principal spot at the *R<sub>f</sub>* value of about 0.4 from the sample solution are not more intense than the spot from the standard solution.

**Giemsa's TS** Dissolve 3 g of azure II-eosin Y 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 Y is prepared by coupling eosin Y to azure II. Azure II is the mixture of equal quantities of methylene azure (azure I), prepared by oxidizing methylene blue, and methylene blue.

**Giemsa's TS, dilute** See Test Methods for Plastic Containers <7.02>.

**[6]-Gingerol for assay** [6]-Gingerol for thin-layer chromatography. However, it meets the following requirements:  
**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (281 nm): 101 – 112 [7 mg, ethanol (99.5), 200 mL].

**Purity** Related substances—Dissolve 5 mg of [6]-gingerol for assay in 5 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than [6]-gingerol from the sample solution is not larger than the peak area of [6]-gingerol from the standard solution.

#### Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Hangekobokuto Extract.

Time span of measurement: About 6 times as long as the retention time of [6]-gingerol.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of [6]-gingerol obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

**[6]-Gingerol for component determination** See [6]-gingerol for assay.

**[6]-Gingerol for thin-layer chromatography**  $C_{17}H_{26}O_4$   
A yellow-white to yellow, liquid or solid. Freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution of [6]-gingerol for thin-layer chromatography in ethanol (99.5) (7 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 279 nm and 283 nm.

**Purity** Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.3 does not appear.

**Ginsenoside Rc**  $C_{53}H_{90}O_{22} \cdot xH_2O$  A white crystalline powder. It is odorless.

**Purity**—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Rc and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

**Ginsenoside Re**  $C_{48}H_{82}O_{18} \cdot xH_2O$  A white crystalline powder. It is odorless.

**Purity**—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Re and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

**Ginsenoside Rg<sub>1</sub> for thin-layer chromatography**  $C_{42}H_{72}O_{14}$  White, crystalline powder, having a slight, bitter taste. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether and in chloroform.

**Melting point** <2.60>: 194 – 196.5°C

**Purity** Related substances—Dissolve 1 mg of ginsenoside Rg<sub>1</sub> for thin layer chromatography in 1 mL of methanol, and perform the test with 20  $\mu$ L of this solution as directed in the Identification (2) under Ginseng: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Glacial acetic acid** See acetic acid (100).

**Glacial acetic acid for nonaqueous titration** See acetic acid for nonaqueous titration.

**Glacial acetic acid-sulfuric acid TS** See acetic acid (100)-sulfuric acid TS.

**$\gamma$ -Globulin** A plasma protein obtained from human serum as Cohn's II and III fractions. White crystalline powder. It contains not less than 98% of  $\gamma$ -globulin in the total protein.

**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 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> 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 detection TS** Dissolve 1600 units of glucose oxidase, 16 mg of 4-aminoantipyrine, 145 units of peroxidase and 0.27 g of *p*-hydroxybenzoic acid in tris buffer solution, pH 7.0, to make 200 mL.

**Glucose detection TS for penicillium origin  $\beta$ -galactosidase** Dissolve glucose oxidase (not less than 500 units), peroxidase (not less than 50 units), 0.01 g of 4-aminoantipyrine and 0.1 g of phenol in phosphate buffer, pH 7.2 to make 100 mL.

**Glucose oxidase** Obtained from *Aspergillus nigar*. White powder. It is freely soluble in water. It contains about 200 Units per mg. One unit indicates an amount of the enzyme which produces 1  $\mu$ mol of D-glucono- $\delta$ -lactone in 1 minute at 25°C and pH 7.0 from glucose used as the substrate.

**Glucose-pepton medium for sterility test** See soybean-casein digest medium

**Glucose TS** Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

**4'-O-Glucosyl-5-O-methylvisamminol for thin-layer chromatography**  $C_{22}H_{28}O_{10}$  White crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water.

**Identification**—Determine the absorption spectrum of a solution of 4'-O-glucosyl-5-O-methylvisamminol for thin-layer chromatography in ethanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 286 nm and 290 nm.

**Purity** Related substances—Dissolve 1 mg of 4'-O-glucosyl-5-O-methylvisamminol for thin-layer chromatography in 1 mL of methanol. Perform the test with 5  $\mu$ L of this solution directed in the Identification under Saposhnikovia Root: no spots other than the principal spot at around *Rf* value of 0.3 appears.

**L-Glutamic acid**  $HOOC(CH_2)_2CH(NH_2)COOH$   
[K 9047, Special class]

**L-Glutamine**  $H_2NCO(CH_2)_2CH(NH_2)COOH$   
[K 9103, Special class]

**Glutamine TS** See Test Methods for Plastic Containers <7.02>.

**7-(Glutarylglucyl-L-arginylamino)-4-methylcoumarin**  $C_{23}H_{30}N_6O_7$  White powder. It is freely soluble in acetic acid

(100), sparingly soluble in dimethylsulfoxide, and practically insoluble in water.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (325 nm): 310 – 350 [2 mg, diluted acetic acid (100) (1 in 500), 200 mL].

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : –50 – –60° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

**Purity** Related substances—Prepare the sample solution by dissolving 5 mg of 7-(glutarylglucyl-L-arginylamino)-4-methylcoumarin in 0.5 mL of acetic acid (100), and perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, allow the plate to stand for 30 minutes in a box filled with iodine vapors: any observable spot other than the principal spot at the *Rf* value of about 0.6 does not appear.

**7-(Glutarylglucyl-L-arginylamino)-4-methylcoumarin TS** Dissolve 5 mg of 7-(glutarylglucyl-L-arginylamino)-4-methylcoumarin in 0.5 to 1 mL of acetic acid (100), lyophilize, dissolve this in 1 mL of dimethylsulfoxide, and use this solution as solution A. Dissolve 30.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.6 g 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 1 mL of the solution A and 500 mL of the solution B before use.

**Glutathione**  $C_{10}H_{17}N_3O_6S$  [Same as the namesake monograph]

**Glycerin**  $C_3H_8O_3$  [K 8295, Glycerol, Special class. Same as the monograph Concentrated Glycerin]

**85% Glycerin**  $C_3H_8O_3$  [Same as the monograph Glycerin]

**Glycine**  $C_2H_6NO_2$  [K 8291, Special class]

**Glycolic acid**  $C_2H_4O_3$  **Purity:** not less than 98.0%.

**Glycyrrhizic acid for thin-layer chromatography**  $C_{42}H_{62}O_{16} \cdot xH_2O$  Colorless or white, sweet, crystalline powder. Freely soluble in hot water and in ethanol (95), and practically insoluble in diethyl ether. Melting point: 213 – 218°C (with decomposition).

**Purity** Related substances—Dissolve 10 mg of glycyrrhizic acid for thin-layer chromatography in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Glycyrrhiza: the spots other than the principal spot at the *Rf* value of about 0.3 from the sample solution are not more intense than the spot from the standard solution.

**Goat anti-ECP antibody** Combine 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and immunize goats subcutaneously in the back region with this solution 5 times at 2 week intervals. Harvest blood on the 10<sup>th</sup> day after completing the immunization to obtain goat antiserum. Goat anti-ECP antibody is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to

sepharose 4B and then purifying by affinity column chromatography.

*Description:* Clear and colorless solution.

*Identification:* When sodium lauryl sulfate-supplemented polyacrylamide gel electrophoresis is conducted under non-reducing conditions, the molecular weight of the major band is within the range of  $1.30 \times 10^5$  to  $1.70 \times 10^5$ .

*Protein content:* When determining the protein content using Assay (1) under CelmoLeukin (Genetical Recombination), the protein content per mL is 0.2 to 1.0 mg.

**Goat anti-ECP antibody TS** Dilute goat anti-ECP antibody with 0.1 mol/L carbonate buffer solution, pH 9.6 to prepare a solution containing 50  $\mu$ g protein per mL.

**Griess-Romijn'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-Romijn'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**  $\text{CH}_3\text{OC}_6\text{H}_4\text{OH}$  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  $\mu$ L of guaiacol as directed under Gas Chromatography <2.02> 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: It showed the purity of not less than 99.0%.

*Operating conditions*

Detector: 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- $\mu$ m 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  $\mu$ L of guaiacol is about 90% of the full scale.

Time span of measurement: About 3 times as long as the retention time of guaiacol beginning after the solvent peak.

**Guaiacol for assay**  $\text{C}_7\text{H}_8\text{O}_2$  Colorless to yellow clear liquid or colorless crystals with a characteristic, aromatic odor. Miscible with methanol and with ethanol (99.5), and sparingly soluble in water. Congealing point: 25 – 30°C.

*Identification*—Determine the infrared absorption spectrum of guaiacol for assay as directed in the ATR method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1595  $\text{cm}^{-1}$ , 1497  $\text{cm}^{-1}$ , 1443  $\text{cm}^{-1}$ , 1358  $\text{cm}^{-1}$ , 1255  $\text{cm}^{-1}$ , 1205  $\text{cm}^{-1}$ , 1108  $\text{cm}^{-1}$ , 1037  $\text{cm}^{-1}$ , 1020  $\text{cm}^{-1}$ , 916  $\text{cm}^{-1}$ , 833  $\text{cm}^{-1}$ , and 738  $\text{cm}^{-1}$ .

*Purity* Related substances—Perform the test with 0.5  $\mu$ L of guaiacol for assay as directed under Gas Chromatography

<2.02> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of guaiacol is not more than 2.0%.

*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  $\mu$ m in thickness.

Column temperature: Raise the temperature from 100°C 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 guaiacol is about 8 minutes.

Split ratio: 1:50.

*System suitability*

Test for required detectability: Weigh accurately about 70 mg of guaiacol for assay, add methanol to make exactly 100 mL, and use this solution for the solution for system suitability test. Confirm that the peak area of guaiacol obtained from 1  $\mu$ L of the solution for system suitability test is equivalent to 0.08 to 0.16% of that of guaiacol obtained when 0.5  $\mu$ L of guaiacol for assay is injected.

System performance: When the procedure is run with 1  $\mu$ L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of guaiacol are not less than 200,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 1  $\mu$ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 2.0%.

**Guaiifenesin**  $\text{C}_{10}\text{H}_{14}\text{O}_4$  [Same as the namesake monograph]

**Guanine**  $\text{C}_5\text{H}_5\text{N}_5\text{O}$  White to pale yellowish white powder.

*Absorbance* <2.24> Weigh accurately about 10 mg of guanine, dissolve in 20 mL of dilute sodium hydroxide TS, and add 2 mL of 1 mol/L hydrochloric acid TS and 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL. Determine the absorbances,  $E_{1\%}^{1\text{cm}}$ , of this solution at 248 nm and 273 nm: they are between 710 and 770, and between 460 and 500, respectively.

*Loss on drying* <2.41>: Not more than 1.5% (0.5 g, 105°C, 4 hours).

**Haloperidol for assay**  $\text{C}_{21}\text{H}_{23}\text{ClFNO}_2$  [Same as the monograph Haloperidol]

**Hanus' TS** Dissolve 20 g of iodine monobromide in 1000 mL of acetic acid (100). Preserve in light-resistant, glass-stoppered bottles, in a cold place.

**Heart infusion agar medium** Prepared for biochemical tests.

**Heavy hydrogenated solvent for nuclear magnetic resonance spectroscopy** Prepared for nuclear magnetic resonance spectroscopy. Heavy hydrogenated chloroform ( $\text{CDCl}_3$ ), heavy hydrogenated dimethyl sulfoxide [ $(\text{CD}_3)_2$

SO], heavy water (D<sub>2</sub>O), and heavy hydrogenated pyridine (C<sub>5</sub>D<sub>5</sub>N) are available.

**Heavy water for nuclear magnetic resonance spectroscopy** D<sub>2</sub>O Prepared for nuclear magnetic resonance spectroscopy.

**Helium** He Not less than 99.995 vol%.

**Hematoxylin** C<sub>16</sub>H<sub>14</sub>O<sub>6</sub>.nH<sub>2</sub>O 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* <2.44>: not more than 0.1% (1 g).

**Hematoxylin TS** Dissolve 1 g of hematoxylin in 12 mL of ethanol (99.5). Dissolve 20 g of aluminum potassium sulfate 12-water 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]

**HEPES buffer solution, pH 7.5** Dissolve 2.38 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 90 mL of water, adjust to pH 7.5 with diluted 6 mol/L sodium hydroxide TS (5 in 6), and add water to make 100 mL.

**Heptane** CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub> [K 9701, Special class]

**Heptane for liquid chromatography** C<sub>7</sub>H<sub>16</sub> Clear and colorless solution.

*Purity* Ultraviolet-absorbing substances—Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances at 210 nm, 220 nm, 230 nm and 240 nm, using water as the control solution: the absorbance is not more than 0.35, not more than 0.15, not more than 0.05 and not more than 0.03, respectively.

**Heptyl parahydroxybenzoate** C<sub>14</sub>H<sub>20</sub>O<sub>3</sub> White crystals or crystalline powder.

*Melting point* <2.60>: 45 – 50°C

*Content*: Not less than 98.0% Assay—Weigh accurately about 3.5 g of heptyl parahydroxybenzoate, dissolve in 50 mL of diluted *N,N*-dimethylformamide (4 in 5), and titrate <2.50> with 1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS  
= 236.3 mg of C<sub>14</sub>H<sub>20</sub>O<sub>3</sub>

**Hesperidin for assay** C<sub>28</sub>H<sub>34</sub>O<sub>15</sub> Hesperidin for thin-layer chromatography. It meets the following requirement.

*Optical rotation* <2.49> [α]<sub>D</sub><sup>20</sup>: –100 – –120° (5 mg dried with silica gel for 24 hours, methanol, 50 mL, 100 mm).

*Purity* Related substances—Dissolve 2 mg of hesperidin for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, 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 <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of hesperidin and the solvent is not larger than the peak area of hesperidin obtained with the

standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Hochuekkito Extract.

Time span of measurement: About 6 times as long as the retention time of hesperidin.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of hesperidin obtained with 10 μL of this solution is equivalent to 3.5% to 6.5% of that with 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Hochuekkito Extract.

**Hesperidin for component determination** See hesperidin for assay.

**Hesperidin for thin-layer chromatography** C<sub>28</sub>H<sub>34</sub>O<sub>15</sub> A white to light brown-yellow, crystalline powder or powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 245°C (with decomposition).

*Absorbance* <2.24> *E*<sub>1</sub><sup>1%</sup><sub>cm</sub> (284 nm): 310 – 340 (8 mg dried in a desiccator (silica gel) for 24 hours, methanol, 500 mL).

*Purity* Related substances—Dissolve 1 mg in 2 mL of methanol. Proceed the test with 20 μL of this solution as directed in the Identification (6) under Hochuekkito Extract: no spot other than the principle spot of around *R*<sub>f</sub> 0.3 appears.

**Hexaammonium heptamolybdate-cerium (IV) sulfate TS** Dissolve 2.5 g of hexaammonium heptamolybdate tetrahydrate and 1.0 g of cerium (IV) sulfate tetrahydrate in diluted sulfuric acid (3 in 50) to make 100 mL. Prepare before use.

**Hexaammonium heptamolybdate-sulfuric acid TS** Dissolve 1.0 g of hexaammonium heptamolybdate tetrahydrate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

**Hexaammonium heptamolybdate tetrahydrate** (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O [K 8905, Special class]

**Hexaammonium heptamolybdate TS** dissolve 21.2 g of hexaammonium heptamolybdate tetrahydrate in water to make 200 mL (10%). Prepare before use.

**Hexamethylenetetramine** (CH<sub>2</sub>)<sub>6</sub>N<sub>4</sub> [K 8847, Special class]

**Hexamethylenetetramine TS** Dissolve 2.5 g of hexamethylenetetramine in 25 mL of water.

**Hexamine** See hexamethylenetetramine.

**Hexane** C<sub>6</sub>H<sub>14</sub> [K 8848, Special class]

**Hexane for liquid chromatography** CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub> Colorless, clear liquid. Miscible with ethanol (95), with diethyl ether, with chloroform and with benzene.

*Boiling point* <2.57>: about 69°C

*Purity* (1) Ultraviolet absorptive substances—Read the absorbances of hexane for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: not more than 0.3 at the wave-

length of 210 nm, and not more than 0.01 between 250 nm and 400 nm.

(2) Peroxide—To a mixture of 100 mL of water and 25 mL of dilute sulfuric acid add 25 mL of a solution of potassium iodide (1 in 10) and 20 g of hexane for liquid chromatography. Stopper tightly, shake, and allow to stand in a dark place for 15 minutes. Titrate <2.50> this solution, while shaking well, with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

**n-Hexane for liquid chromatography** See hexane for liquid chromatography.

**Hexane for purity of crude drug** [K 8848, Special class] Use hexane meeting the following additional specification. Evaporate 300.0 mL of hexane for purity of crude drug in vacuum at a temperature not higher than 40°C, add the hexane to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of  $\gamma$ -BHC in hexane to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with exactly 1  $\mu$ L each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peak other than the solvent peak from the sample solution is not larger than the peak area of  $\gamma$ -BHC from the standard solution I.

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of  $\gamma$ -BHC obtained from 1  $\mu$ L of the standard solution II can be measured by the automatic integration method, and the peak height of  $\gamma$ -BHC from 1  $\mu$ L of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of  $\gamma$ -BHC beginning after the solvent peak.

**Hexane for ultraviolet-visible spectrophotometry** [K 8848, Special class]. When determining the absorbance of hexane for ultraviolet-visible spectrophotometry as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution, its value is not more than 0.10 at 220 nm and not more than 0.02 at 260 nm, and it has no characteristic absorption between 260 nm and 350 nm.

**n-Hexane for ultraviolet-visible spectrophotometry** See hexane for ultraviolet-visible spectrophotometry.

**Hexyl parahydroxybenzoate**  $C_{13}H_{18}O_3$  White crystals or crystalline powder.

*Melting point* <2.60>: 49 – 53°C

*Content*: Not less than 98.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS  
= 222.3 mg of  $C_{13}H_{18}O_3$

**Hirsutine** See hirsutine for thin-layer chromatography.

**Hirsutine for assay**  $C_{22}H_{28}N_2O_3$  Hirsutine for thin-layer chromatography. It meets the following requirements.

*Absorbance* <2.24>:  $E_{1\text{cm}}^{1\%}$  (245 nm): 354 – 389 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

*Purity* Related substances—Dissolve 5 mg of hirsutine for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid-chromatography <2.01> according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than hirsutine obtained from the sample solution is not larger than the peak area of hirsutine from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 1.5 times as long as the retention time of hirsutine, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of hirsutine obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay under Uncaria Hook.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hirsutine is not more than 1.5%.

**Hirsutine for thin-layer chromatography**  $C_{22}H_{28}N_2O_3$  A white or light orange crystalline powder or powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 105°C.

*Identification*—Determine the absorption spectrum of a solution of hirsutine for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 287 nm and 291 nm.

*Purity* Related substances—Dissolve 1.0 mg of hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than the principal spot at around  $R_f$  0.55 appears.

**L-Histidine**  $C_6H_9N_3O_2$  [Same as the namesake monograph]

**L-Histidine hydrochloride** See L-histidine hydrochloride monohydrate.

**L-Histidine hydrochloride monohydrate**  
 $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$  [K 9050, Special class]

**Homatropine hydrobromide**  $C_{16}H_{21}NO_3 \cdot HBr$  [Same as the namesake monograph]

**Honokiol**  $C_{18}H_{18}O_2 \cdot xH_2O$  Odorless white, crystals or crystalline powder.

**Purity**—Dissolve 1 mg of honokiol in the mobile phase to make 10 mL, and use this solution as the sample solution. Perform the Liquid Chromatography <2.01> with 10  $\mu$ L of the sample solution as directed in the Assay under Magnolia Bark: when measure the peak areas 2 times as long as the retention time of magnorole, the total area of peaks other than honokiol is not larger than 1/10 times the total area of the peaks other than the solvent peak.

**Horseradish peroxidase** An oxidase (Molecular weight: about 40,000) derived from horseradish.

**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  $-20^\circ\text{C}$ .

**Human antithrombin III** Serine protease inhibition factor obtained from normal plasma of health human. It is a protein, which inhibits the activities of thrombin and activated blood coagulation factor X. It contains not less than 300 Units per mg protein. One unit indicates an amount of the antithrombin III which inhibits 1 unit of thrombin at  $25^\circ\text{C}$  under the existence of heparin.

**Human chorionic gonadotrophin TS** Weigh accurately a suitable amount of Human Chorionic Gonadotrophin according to the labeled amount, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2 so that each mL contains 80 human chorionic gonadotrophin Units.

**Human insulin desamide substance-containing TS** Dissolve 1.5 mg of Insulin Human in 1 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at  $25^\circ\text{C}$  for 3 days, and when the procedure is run with this solution according to the conditions as directed in the Purity (1) under Insulin Human (Genetical Recombination), the solution contains about 5% of the desamide substance.

**Human insulin dimer-containing TS** Allow to stand Insulin Human (Genetical Recombination) at  $25^\circ\text{C}$  for 10 days or more, and dissolve 4 mg of this in 1 mL of 0.01 mol/L hydrochloric acid TS.

**Human normal plasma** Dissolve an amount of dried human normal plasma powder, equivalent to 1 mL of the normal plasma of human, in 1 mL of water. Store between  $2$  and  $10^\circ\text{C}$ , and use within one week.

**Human serum albumin for assay** White to pale-yellow powder. Albumin content is at least 99%. Convert to the dehydrate using the following water determination method.

Water content <2.48>: (0.2 g, volumetric titration, direct titration). However, in a dehydration solvent, use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1).

**Hydralazine hydrochloride**  $C_8H_8N_4 \cdot HCl$  [Same as the namesake monograph]

**Hydralazine hydrochloride for assay**  $C_8H_8N_4 \cdot HCl$   
 [Same as the monograph Hydralazine Hydrochloride. When dried, it contains not less than 99.0% of hydralazine hydrochloride ( $C_8H_8N_4 \cdot HCl$ ).]

**Hydrazine monohydrate**  $NH_2NH_2 \cdot H_2O$  Colorless liquid, having a characteristic odor.

**Hydrazine sulfate** See hydrazinum sulfate.

**Hydrazinum sulfate**  $N_4H_6SO_4$  [K 8992, Special class]

**Hydrazinum sulfate TS** Dissolve 1.0 g of hydrazinum sulfate in water to make 100 mL.

**Hydrobromic acid** HBr [K 8509, Special class]

**Hydrochloric acid** HCl [K 8180, Special class]

**Hydrochloric acid-ammonium acetate buffer solution, pH 3.5** Dissolve 25 g of ammonium acetate in 45 mL of 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

**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 to make 100 mL.

**0.01 mol/L Hydrochloric acid-methanol TS** To 20 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

**0.05 mol/L Hydrochloric acid-methanol TS** To 100 mL of 0.5 mol/L hydrochloric acid add methanol to make 1000 mL.

**Hydrochloric acid-2-propanol TS** Add 0.33 mL of hydrochloric acid to 100 mL of 2-propanol, mix, and store in a dark and cool place.

**Hydrochloric acid-potassium chloride buffer solution, pH 2.0** To 10.0 mL of 0.2 mol/L hydrochloric acid VS add 88.0 mL of 0.2 mol/L potassium chloride TS, adjust the pH to  $2.0 \pm 0.1$  with 0.2 mol/L hydrochloric acid VS or 0.2 mol/L potassium chloride TS, 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 hydro-

chloric 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.

**10 mol/L Hydrochloric acid TS** Dilute 900 mL of hydrochloric acid with water to make 1000 mL.

**Hydrocortisone**  $C_{21}H_{30}O_5$  [Same as the namesake monograph]

**Hydrocortisone acetate**  $C_{23}H_{32}O_6$  [Same as the namesake monograph]

**Hydrocotarnine hydrochloride for assay** See hydrocotarnine hydrochloride hydrate for assay.

**Hydrocotarnine hydrochloride hydrate for assay**  $C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$  [Same as the monograph Hydrocotarnine Hydrochloride Hydrate. When dried, it contains not less than 99.0% of hydrocotarnine hydrochloride ( $C_{12}H_{15}NO_3 \cdot HCl$ ).]

**Hydrofluoric acid** HF [K 8819, Special class] It contains not less than 46.0% of HF.

**Hydrogen**  $H_2$  [K 0512, Standard substance, Third class] It contains not less than 99.99% of  $H_2$ .

**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 dropwise 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 hexachloroplatinate (IV) hexahydrate**  $H_2PtCl_6 \cdot 6H_2O$  [K 8153, Special class]

**Hydrogen hexachloroplatinate (IV)-potassium iodide TS** To 3 mL of hydrogen hexachloroplatinate (IV) TS add 97 mL of water and 100 mL of a solution of potassium iodide (3 in 50). Prepare before use.

**Hydrogen hexachloroplatinate (IV) TS** Dissolve 2.6 g of chloroplatinic acid in water to make 20 mL (0.25 mol/L).

**Hydrogen peroxide (30)**  $H_2O_2$  [K 8230, Hydrogen peroxide, Special class, Concentration: 30.0 – 35.5%.]

**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 1 mL of hydrogen peroxide (30) with 500 mL of water, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

**Hydrogen peroxide water, strong** See hydrogen peroxide (30).

**Hydrogen sulfide**  $H_2S$  Colorless, 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.

**Hydrogen tetrachloroaurate (III) tetrahydrate**  $HAuCl_4 \cdot 4H_2O$  [K 8127, Special class]

**Hydrogen tetrachloroaurate (III) TS** Dissolve 1 g of hydrogen tetrachloroaurate (III) tetrahydrate in 35 mL of water (0.2 mol/L).

**Hydroiodic acid** HI [K 8917, Special class]

**Hydroquinone**  $C_6H_4(OH)_2$  [K 8738, Special class]

**Hydroxocobalamin acetate**  $C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2$  Dark red crystals or powder.

*Loss on drying* <2.41>: not more than 12% (50 mg, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 6 hours).

*Content*: not less than 98.0%. Assay—Proceed as directed in the Assay under Hydroxocobalamin Acetate.

***m*-Hydroxyacetophenone**  $C_8H_8O_2$  White to light yellowish white crystalline powder.

*Melting point* <2.60>: about 96°C

*Purity* Related substances—Perform the test with 10  $\mu$ L of a solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000) as directed in the Assay under Cefalexin: Any obstructive peaks for determination of cefalexin are not observed.

***p*-Hydroxyacetophenone**  $C_8H_8O_2$  White to pale yellow crystals or crystalline powder. It is freely soluble in methanol.

*Melting point* <2.60>: 107 – 111°C

*Purity*—Weigh 1 mg of *p*-hydroxyacetophenone, add methanol and dissolve to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution as directed under Liquid Chromatography <2.01> according to the Assay under Peony Root: the total area of the peaks other than the peak of *p*-hydroxyacetophenone from the sample solution is not larger than 3/100 of the total area of the peaks other than the solvent peak.

**3-Hydroxybenzoic acid**  $HOC_6H_4COOH$  White, crys-

tals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum according to the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3300\text{ cm}^{-1}$ ,  $1690\text{ cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1307\text{ cm}^{-1}$ ,  $1232\text{ cm}^{-1}$  and  $760\text{ cm}^{-1}$ .

**Melting point** <2.60>:  $203 - 206^\circ\text{C}$

**Purity** Clarity—Dissolve 1 g of 3-hydroxybenzoic acid in 20 mL of methanol: the solution is clear.

**Content**: not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 3-hydroxybenzoic acid, dissolve in 20 mL of diluted ethanol (95) (1 in 2), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of cresol red TS) until the color of the solution changes from yellow to dark orange-red. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 13.81 mg of  $\text{C}_7\text{H}_6\text{O}_3$

***p*-Hydroxybenzoic acid** See parahydroxybenzoic acid.

**10-Hydroxy-2-(*E*)-decenoic acid for assay** 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—Dissolve 10 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of 10-hydroxy-2-(*E*)-decenoic acid from sample solution is not larger than the peak area of 10-hydroxy-2-(*E*)-decenoic acid from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Royal Jelly.

Time span of measurement: About 4 times as long as the retention time of 10-hydroxy-2-(*E*)-decenoic acid beginning after the solvent peak.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of 10-hydroxy-2-(*E*)-decenoic acid obtained from 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that of 10-hydroxy-2-(*E*)-decenoic acid from 10  $\mu\text{L}$  of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Royal Jelly.

**10-Hydroxy-2-(*E*)-decenoic acid for component determination** See 10-hydroxy-2-(*E*)-decenoic acid for assay

**10-Hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography**  $\text{C}_{10}\text{H}_{18}\text{O}_3$  White crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), soluble in diethyl ether, and slightly soluble in water.

**Identification**—Determine the absorption spectrum of a solution of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer

chromatography in ethanol (99.5) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 206 nm and 210 nm.

**Melting point** <2.60>:  $63 - 66^\circ\text{C}$

**Purity** Related substances—Dissolve 5.0 mg of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in 1 mL of diethyl ether. Perform the test with 20  $\mu\text{L}$  of this solution as directed in the Identification under Royal Jelly: no spot other than the principal spot at around *R<sub>f</sub>* value of 0.5 appears.

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride**  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_3\text{S}\cdot\text{HCl}$  To 9 g of diltiazem hydrochloride add 50 mL of ethanol (99.5), and dissolve by heating at  $80^\circ\text{C}$ . To this solution add slowly 50 mL of a solution of potassium hydroxide in ethanol (99.5) (33 in 500) dropwise, 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 50 mg of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride in chloroform to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu\text{L}$  of the sample 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** <2.48>: not 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(5*H*)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS  
= 40.89 mg of  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_3\text{S}\cdot\text{HCl}$

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride** See *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride.

***N*-(2-Hydroxyethyl)isonicotinamide nitric ester**  $\text{C}_8\text{H}_9\text{N}_3\text{O}_4$  A white crystalline powder.

**Identification**—Determine the infrared absorption spectrum of *N*-(2-hydroxyethyl)isonicotinamide nitric ester as di-

rected in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3270\text{ cm}^{-1}$ ,  $1653\text{ cm}^{-1}$ ,  $1546\text{ cm}^{-1}$  and  $1283\text{ cm}^{-1}$ .

***N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid**  $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_4\text{S}$  White crystalline powder.

**Purity** Clarity and color of solution—Dissolve 11.9 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 50 mL of water: the solution is clear and colorless.

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 1 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, dissolve in 60 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (Potentiometric titration).

Each mL of 0.5 mol/L sodium hydroxide VS  
= 119.2 mg of  $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_4\text{S}$

**1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol**  $\text{C}_3\text{H}_6\text{N}_4\text{OS}$   
White, crystals or powder.

**Melting point** <2.60>:  $136 - 141^\circ\text{C}$

**Purity** Related substances—Dissolve 0.10 g of 1-(2-hydroxyethyl)-1*H*-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 <2.03>. Spot  $1\ \mu\text{L}$  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.

**2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid**  $\text{C}_{21}\text{H}_{14}\text{N}_2\text{O}_7\text{S}$  [K 8776, Special class]

**4-Hydroxyisophthalic acid**  $\text{HOC}_6\text{H}_3(\text{COOH})_2$  White crystals or powder.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.14 g of 4-hydroxyisophthalic acid, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.107 mg of  $\text{C}_8\text{H}_6\text{O}_5$

**Hydroxylamine hydrochloride** See hydroxylammonium chloride.

**Hydroxylamine hydrochloride-ferric chloride TS** See hydroxylammonium chloride-iron (III) chloride TS.

**Hydroxylamine hydrochloride TS** See hydroxylammonium chloride TS.

**Hydroxylamine perchlorate**  $\text{NH}_2\text{OH}.\text{HClO}_4$  Hygroscopic, white crystals. Dissolves in water and in ethanol (95).  
**Melting point** <2.60>:  $87.5 - 90^\circ\text{C}$

**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** An ethanol (99.5) 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, 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** See hydroxylammonium chloride TS, pH 3.1.

**Hydroxylammonium chloride**  $\text{NH}_2\text{OH}.\text{HCl}$  [K 8201, Special class]

**Hydroxylammonium chloride-ethanol TS** Dissolve 34.8 g of hydroxylammonium chloride in water to make 100 mL, and use this solution as Solution A. Dissolve 10.3 g of sodium acetate trihydrate and 86.5 g of sodium hydroxide in water to make 1000 mL, and use this solution as Solution B. Mix 1 volume of Solution A, 1 volume of Solution B and 4 volumes of ethanol (95).

**Hydroxylammonium chloride-iron (III) chloride TS** Acidify 100 mL 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 65 mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia solution (28) until the solution exhibits a yellow color. Shake well after adding 10 mL 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 portions 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 dropwise dilute hydrochloric acid to this aqueous solution until it exhibits a red color, then add water to make 100 mL.

**Hydroxylammonium chloride TS, pH 3.1** Dissolve 6.9 g of hydroxylammonium chloride in 80 mL of water, adjust the pH to 3.1 by adding dilute sodium hydroxide TS, and add water to make 100 mL.

**4-Hydroxy-3-methoxybenzyl nonylic acid amide**  $\text{C}_{17}\text{H}_{27}\text{NO}_3$  A white crystalline powder, having a faint, characteristic odor.

**Purity** Related substances—Dissolve 10 mg in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly  $20\ \mu\text{L}$  each of the sample solution and standard solution as directed in the Assay under Capsicum: when measure the peak areas 2 times as long as the

retention time of capsaicin, the total area of the peaks other than 4-hydroxy-3-methoxybenzyl nonylic acid amide is not larger than the peak area of 4-hydroxy-3-methoxybenzyl nonylic acid amide from the standard solution.

### 3-(3-Hydroxy-4-methoxyphenyl)-2-(E)-propenic acid

$C_{10}H_{10}O_4$  White to light yellow, crystals or crystalline powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 230°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, between 238 nm and 242 nm, between 290 nm and 294 nm, and between 319 nm and 323 nm.

**Purity** Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2  $\mu$ L of this solution as directed in the Identification (11) under Hochuekkito Extract: no spot other than the principle spot of around  $R_f$  0.6 appears.

**3-(3-Hydroxy-4-methoxyphenyl)-2-(E)-propenic acid-(E)-ferulic acid TS for thin-layer chromatography** Dissolve 1 mg of 3-(3-hydroxy-4-methoxyphenyl)-2-(E)-propenic acid and 1 mg of (E)-ferulic acid in 2 mL of methanol.

**2-[4-(2-Hydroxymethyl)-1-piperazinyl] propanesulfonic acid**  $C_8H_{18}N_2O_4S$  A white crystalline powder.

**Residue on ignition** <2.44>: not more than 0.1%.

**Content**: not less than 99%.

**N-(3-Hydroxyphenyl)acetamide**  $C_8H_9NO_2$  White to pale yellowish white crystals. It is freely soluble in ethanol (95), and sparingly soluble in water.

**Melting point** <2.60>: 146 – 149°C

**Purity** (1) Clarity and color of solution—Dissolve 0.5 g of *N*-(3-hydroxyphenyl)acetamide in 50 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.1 g of *N*-(3-hydroxyphenyl)acetamide in 1000 mL of water. Pipet 10 mL of this solution, add 6.5 mL of acetonitrile and water to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed in the Assay under Aspoxicillin Hydrate: any peak other than those of *N*-(3-hydroxyphenyl)acetamide and the solvent does not appear.

**3-(*p*-Hydroxyphenyl)propionic acid**  $C_9H_{10}O_3$

**Description**—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)propionic acid, previously dried (in vacuum, 60°C, 4 hours), dissolve in 5 mL of methanol, add 45 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 16.62 mg of  $C_9H_{10}O_3$

### Hyodeoxycholic acid for thin-layer chromatography

$C_{24}H_{40}O_4$  White to pale brown crystalline powder or powder. Freely soluble in methanol in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the infrared absorption spec-

trum of hyodeoxycholic acid for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940  $cm^{-1}$ , 2840  $cm^{-1}$ , 1740  $cm^{-1}$ , 1460  $cm^{-1}$ , 1340  $cm^{-1}$ , 1200  $cm^{-1}$ , 1160  $cm^{-1}$ , 1040  $cm^{-1}$  and 600  $cm^{-1}$ .

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +7 – +10° (0.4 g, ethanol (99.5), 20 mL, 100 mm).

**Melting point** <2.60>: 198 – 205°C

**Purity** Related substances—Dissolve 20 mg of hyodeoxycholic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: the spots other than the principal spot at the  $R_f$  value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

**Hypaconitine for purity**  $C_{33}H_{45}NO_{10}$  White, crystals or crystalline powder. Soluble in acetonitrile, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water. Melting point: about 175°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of hypaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500  $cm^{-1}$ , 1728  $cm^{-1}$ , 1712  $cm^{-1}$ , 1278  $cm^{-1}$ , 1118  $cm^{-1}$ , 1099  $cm^{-1}$  and 714  $cm^{-1}$ .

**Absorbance** <2.24>  $E_{1\%}^{1\text{cm}}$  (230 nm): 217 – 252 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of hypaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of hypaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of hypaconitine and the solvent obtained with the sample solution is not larger than the peak area of hypaconitine with the standard solu-

tion.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of hyaconitine is about 23 minutes.

Time span of measurement: About 3 times as long as the retention time of hyaconitine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of hyaconitine obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10  $\mu$ L of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hyaconitine is not more than 1.5%.

*Water* <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

**Hyperoside for thin-layer chromatography** C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>  
Yellow crystals or crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 220°C (with decomposition).

*Identification*—Determine the absorption spectrum of a solution of hyperoside for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 255 nm and 259 nm.

*Purity* Related substances—Dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under Crataegus Fruit: any spot other than the principal spot of around *R<sub>f</sub>* value of 0.5 does not appear.

**Hypophosphorus acid** See phosphinic acid.

**Hypoxanthine** C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O White crystals or crystalline powder. Freely soluble in ammonia TS, sparingly soluble in dilute hydrochloric acid and in 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 Hydrate: any spot other than the principal spot at the *R<sub>f</sub>* 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 10 mL 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 Ultraviolet-visible Spectrophotometry <2.24>, using phosphate buffer solution, pH 7.0, as the blank solution.

$$\text{Amount (mg) of C}_5\text{H}_4\text{N}_4\text{O} = \frac{A}{779} \times 250,000$$

**Ibuprofen** C<sub>13</sub>H<sub>18</sub>O<sub>2</sub> [Same as the namesake monograph]

**Icariin for thin-layer chromatography** C<sub>33</sub>H<sub>40</sub>O<sub>15</sub> Light yellow crystals. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 234°C (with decomposition).

*Purity* Related substances—Dissolve 1.0 mg of icariin for thin-layer chromatography in 1 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under Epimedium Herb: no spot other than the principal spot having *R<sub>f</sub>* value about 0.4 appears.

**Imidapril hydrochloride** C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>.HCl [Same as the namesake monograph]

**Imidapril hydrochloride for assay** C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>.HCl [Same as the monograph Imidapril Hydrochloride. When dried, it contains not less than 99.0% of imidapril hydrochloride (C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>.HCl).]

**Imidazole** C<sub>3</sub>H<sub>4</sub>N<sub>2</sub> White crystalline powder. Very soluble in water and in methanol.

*Melting point* <2.60>: 89 – 92°C

*Absorbance* <2.24> *E*<sub>1%<sup>1</sup>cm</sub> (313 nm): not more than 0.031 (8 g, water, 100 mL).

**Imidazole for Karl Fischer method** See the Water Determination <2.48>.

**Imidazole for thin-layer chromatography** C<sub>3</sub>H<sub>4</sub>N<sub>2</sub>  
White, crystalline powder. Very soluble in water and in methanol, and freely soluble in ethyl acetate and in dichloromethane.

*Melting point* <2.60>: 89 – 92°C

*Purity* Related substances—Dissolve 10 mg of imidazole for thin-layer chromatography in exactly 20 mL of dichloromethane, and proceed with this solution as directed in the Purity (6) under Clotrimazole: any spot other than the principal spot does not appear.

**Imidazole TS** Dissolve 8.25 g of imidazole in 65 mL of water, adjust the pH to 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 100 mL.

**Iminodibenzyl** C<sub>14</sub>H<sub>13</sub>N White to light brown crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 104 – 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<sub>f</sub>* value of about 0.9 does not appear.

*Nitrogen* <1.08>: 6.8 – 7.3%.

**2,2'-Iminodiethanol hydrochloride**  $C_4H_{11}NO_2 \cdot HCl$ 

A pale yellow liquid.

*Refractive index*  $\langle 2.45 \rangle$   $n_D^{20}$ : 1.515 – 1.519

*Specific gravity*  $\langle 2.56 \rangle$   $d_{20}^{20}$ : 1.259 – 1.263

*Water*  $\langle 2.48 \rangle$ : less than 0.1%.

**Imipramine hydrochloride**  $C_{19}H_{24}N_2 \cdot HCl$  [Same as the namesake monograph]

**Indigo carmine**  $C_{16}H_8N_2Na_2O_8S_2$  [K 8092, Special class]

**Indigo carmine TS** Dissolve 0.20 g of indigo carmine in water to make 100 mL. Use within 60 days.

**2,3-Indolinedione**  $C_8H_5NO_2$  [K 8089, Special class]

**Indometacin**  $C_{19}H_{16}ClNO_4$  [Same as the namesake monograph]

**Insulin human** [Same as the monograph Insulin Human (Genetical Recombination)]

**Interleukin-2 dependent mouse natural killer cell (NKC3)**

Fractionate using discontinuous concentration gradient method cells obtained by removing adhesive cells and phagocytic cells from C3H/He mouse spleen cells. Then, cultivate in soft agar containing interleukin-2 the cell fraction with potent NK activity and obtain the colonies. From among the cell lines obtained, one of the cell lines dependent on interleukin-2 that grows in liquid medium and serially subcultured in liquid medium containing interleukin-2 is identified as NKC3.

**Iodine** I [K 8920, Special class]

**Iodine for assay** I [Same as the monograph Iodine]

**Iodine monobromide** IBr Blackish brown crystals or masses. It dissolves in water, in ethanol (95), in diethyl ether, in carbon disulfide and in acetic acid (100).

*Melting point*  $\langle 2.60 \rangle$ : 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**  $ICl_3$  [K 8403, Special class]

**Iodine TS** Dissolve 14 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid, and dilute with water to make 1000 mL (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 mol/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.

**0.5 mol/L Iodine TS** To 12.7 g of iodine and 25 g of potassium iodide add 10 mL of water, triturate, and add water to make 100 mL.

**Iodoethane**  $C_2H_5I$  A colorless or a dark-brown, clear liquid, having diethyl ether-like odor.

*Distilling range*  $\langle 2.57 \rangle$ : 71.0 – 72.5°C, not less than 94 vol%.

**Iodomethane**  $CH_3I$  [K 8919, Special class]

**Iodomethane for assay**  $CH_3I$  Clear, colorless liquid. On exposure to light, it liberates iodine and becomes brown. Miscible with ethanol (95) and with diethyl ether, and sparingly soluble in water. Use the distillate obtained between 42.2°C and 42.6°C.

*Specific gravity*  $\langle 2.56 \rangle$   $d_{23}^{25}$ : 2.27 – 2.28.

*Purity*—Perform the test with 1  $\mu$ L of iodomethane for assay as directed under Gas Chromatography  $\langle 2.02 \rangle$  according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of iodomethane by the area percentage method: it shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of iodomethane from 1  $\mu$ L of methyl iodide for assay is about 80% of the full scale.

*Content*: not less than 98.0%. Assay—Proceed as directed in the Assay under Isopropyl iodide for assay.

Each mL of 0.1 mol/L silver nitrate VS  
= 14.19 mg of  $CH_3I$

**5-Iodouracil for liquid chromatography**  $C_4H_3IN_2O_2$ 

White, crystalline powder. Melting point: about 275°C (with decomposition).

*Purity*—Dissolve 3 mg of 5-iodouracil for liquid chromatography in a mixture of diluted methanol (1 in 25) to make 10 mL. Perform the test with 10  $\mu$ L of this solution as directed under Liquid Chromatography  $\langle 2.01 \rangle$ , according to the operating conditions in the Purity under Idoxuridine Eye Drops. Measure each peak area by the automatic integration method over a time span of twice as long as the retention time of the principal peak, and calculate the amount of 5-iodouracil by the area percentage method: It shows the purity of not less than 98.5%.

*Content*: not less than 98.5%. Assay—Weigh accurately about 5 mg of 5-iodouracil for liquid chromatography, previously dried at 60°C for 3 hours under reduced pressure, dissolve in water to make exactly 250 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry  $\langle 2.24 \rangle$ , and determine the absorbance  $A$  at the wavelength of maximum absorption at about 282 nm.

Amount (mg) of 5-iodouracil ( $C_4H_3IN_2O_2$ )  
=  $\frac{A}{265} \times 2500$

**Iotalamic acid for assay**  $C_{11}H_9I_3N_2O_4$  [Same as the monograph Iotalamic Acid]

**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 (II) sulfate heptahydrate**  $FeSO_4 \cdot 7H_2O$  [K 8978, 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**  $FeS$  [K 8948, 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 (II) trisodium pentacyanoamine TS** To 1.0 g of sodium pentacyanonitrosylferrate (III) dihydrate add 3.2 mL of ammonia water, shake, stopper closely, and allow to stand in a refrigerator overnight. Add this solution to 10 mL of ethanol (99.5), filter a yellow colored precipitate by suction, wash with ether (99.5), dry, and preserve in a desiccator. Before using, dissolve in water to make a solution of 1.0 mg/mL, and store in a refrigerator. Use within 7 days after preparation.

**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**  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  [K 8142, Special class]

**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, anhydrous** 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 8 mL 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**  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  [K 8559, 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 TS** Dissolve 0.8 g of iron (III) perchlorate hexahydrate in perchloric acid-ethanol TS to make 100 mL.

*Storage*—Preserve in tight containers, in a cold place.

**Iron (III) perchlorate hexahydrate**  $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$  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**  $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$  [K 8981, Special class]

**Iron (III) sulfate TS** Dissolve 50 g of iron (III) sulfate *n*-hydrate in an excess of water, and add 200 mL of sulfuric acid and water to make 1000 mL.

**Iron-phenol TS** Dissolve 1.054 g of ammonium iron (II) sulfate hexahydrate in 20 mL of water, add 1 mL of sulfuric acid and 1 mL of hydrogen peroxide (30), heat until effervescence ceases, and dilute with water to make 50 mL. To 3 volumes of this solution 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 stand in a dark place for 16 to 24 hours. To the mixture add diluted sulfuric acid (10 in 21) equivalent to 23.5% of 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** To 10 mL of iron-phenol TS add 4.5 mL of water. Prepare before use.

**Iron powder** Fe A lusterless, gray to grayish black powder, being attracted by a magnet.

*Identification*—To 1 mL of a solution in hydrochloric acid (1 in 50) add water to make 15 mL, and add 0.1 mL of potassium hexacyanoferrate (III) TS: a blue color appears.

**Iron salicylate TS** Dissolve 0.1 g of ammonium iron (III) sulfate dodecahydrate 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.

**Irsogladine maleate**  $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5 \cdot \text{C}_4\text{H}_4\text{O}_4$  [Same as the namesake monograph]

**Irsogladine maleate for assay**  $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5 \cdot \text{C}_4\text{H}_4\text{O}_4$  [Same as the monograph Irsogladine Maleate. When dried, it contains not less than 99.5% of irsogladine maleate ( $\text{C}_9\text{H}_7\text{Cl}_2\text{N}_5 \cdot \text{C}_4\text{H}_4\text{O}_4$ ).]

**Isatin** See 2,3-indolinedione.

**Isoamyl acetate** See 3-methylbutyl acetate.

**Isoamyl alcohol** See 3-methyl-1-butanol.

**Isoamyl benzoate**  $\text{C}_{12}\text{H}_{16}\text{O}_2$   
*Specific gravity* <2.56>  $d_4^{15}$ : 0.993  
*Boiling point* <2.57>: 260 – 262°C

**Isoamyl parahydroxybenzoate**  $\text{C}_{12}\text{H}_{16}\text{O}_3$  White crystalline powder, having a faint characteristic odor.

It is very soluble in acetonitrile, in ethanol (95), in acetone and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 62 – 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 and in diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 75 – 77°C

*Residue on ignition* <2.44>: not more than 0.1%.

*Content*: not less than 98.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS  
= 194.2 mg of  $C_{11}H_{14}O_3$

**Isobutyl salicylate**  $C_{11}H_{14}O_3$  Colorless, clear liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.506 – 1.511

*Specific gravity* <2.56>  $d_4^{20}$ : 1.068 – 1.073

*Boiling point* <2.57>: 260 – 262°C

*Purity*—Perform the test with 1  $\mu$ L of isobutyl salicylate as directed under Gas Chromatography <2.02> 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: It shows the purity of 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  $\mu$ m in particle diameter, coated with polyethylene 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  $\mu$ L of the sample solution is about 60% to 80% of the full scale.

Time span of measurement: About 3 times as long as the retention time of isobutyl salicylate beginning after the solvent peak.

**Isoelectric point markers for teceleukin** Dissolve 0.02 to 0.05 mg of cytochrome C, trypsinogen, lentil-lectin basic band, lentil-lectin middle band, lentil-lectin acidic band, horse myoglobin basic band, horse myoglobin acidic band, human carbonic anhydrase B, bovine carbonic anhydrase B, and  $\beta$ -lactoglobulin A, in 0.1 mL of saccharose solution (3 in 10).

**L-Isoleucine**  $C_6H_{13}NO_2$  [Same as the namesake monograph]

**L-Isoleucine for assay**  $C_6H_{13}NO_2$  [Same as the monograph L-Isoleucine. When dried, it contains not less than 99.0% of L-isoleucine ( $C_6H_{13}NO_2$ ).]

**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 amide**  $C_6H_6N_2O$  White, crystals or crystalline powder.

*Melting point* <2.60>: 155 – 158°C

*Purity* Clarity of solution—Dissolve 1.0 g of the substance to be tested in 20 mL of methanol: the solution is clear.

*Content*: not less than 99.0%. Assay—Weigh accurately about 0.3 g of isonicotinic acid amide, previously dried, and dissolve in 20 mL of acetic acid (100) by heating. After cooling, add 100 mL of benzene, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 11.21 mg of  $C_6H_6N_2O$

**Isooctane** See octane, iso.

**Isopromethazine hydrochloride for thin-layer chromatography**  $C_{17}H_{20}N_2S.HCl$  White, odorless, crystalline powder. Freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

*Melting point* <2.60>: 193 – 197°C

*Purity* Related substances—Dissolve 5.0 mg of isopromethazine hydrochloride for thin-layer chromatography in exactly 25 mL of ethanol, and perform the test with this solution as directed in the Purity (3) under Promethazine Hydrochloride: any spot other than the principal spot at the  $R_f$  value of about 0.65 does not appear.

**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 *p*-aminobenzoate** See isopropyl 4-aminobenzoate.

**Isopropyl 4-aminobenzoate**  $NH_2C_6H_4COOCH(CH_3)_2$   
Pale brown crystals.

*Melting point* <2.60>: 83 – 86°C

**Isopropyl benzoate**  $C_6H_5COOCH(CH_3)_2$  A clear, colorless liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.490 – 1.498

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.008 – 1.016

**Isopropylether** See propylether, iso.

**Isopropyl *p*-hydroxybenzoate** See isopropyl parahydroxybenzoate.

**Isopropyl iodide for assay**  $C_3H_7I$  Colorless, clear liquid. On exposure to light it liberates iodine and becomes brown. Miscible with ethanol (95), with diethyl ether and with petroleum benzine, and not miscible with water. Use the distillate obtained between 89.0°C and 89.5°C.

*Specific gravity* <2.56>  $d_4^{20}$ : 1.700 – 1.710

**Purity**—Perform the test with 1  $\mu\text{L}$  of isopropyl iodide for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of isopropyl iodide by the area percentage method: It shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of isopropyl iodide from 1  $\mu\text{L}$  of isopropyl iodide for assay is about 80% of the full scale.

**Content**: not less than 98.0%. **Assay**—Transfer 10 mL of ethanol (95) into a brown volumetric flask, weigh accurately, add 1 mL of isopropyl iodide for assay, and weigh accurately again. Add ethanol (95) to make exactly 100 mL, pipet 20 mL of this solution into the second brown volumetric flask, add exactly 50 mL of 0.1 mol/L silver nitrate VS and then 2 mL of nitric acid, stopper, shake occasionally for 2 hours in a dark place, and allow to stand overnight in a dark place. Shake occasionally for 2 hours, add water to make exactly 100 mL, and filter through dry filter paper. Discard the first 20 mL of the filtrate, pipet the next 50 mL, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L silver nitrate VS  
= 17.00 mg of  $\text{C}_3\text{H}_7\text{I}$

**Isopropyl myristate**  $\text{C}_{17}\text{H}_{34}\text{O}_2$  Colorless, clear, oily liquid, and odorless. Congeals at about 5°C. Soluble in 90% alcohol, miscible with many organic solvents and with solid oils, and insoluble in water, in glycerin and in propylene glycol.

*Refractive index* <2.45>  $n_D^{20}$ : 1.432 – 1.436

*Specific gravity* <2.56>  $d_4^{20}$ : 0.846 – 0.854

*Saponification value* <1.13>: 202 – 212

*Acid value* <1.13>: not more than 1.

*Iodine value* <1.13>: not more than 1.

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

**Isopropyl myristate for sterility test**  $\text{C}_{17}\text{H}_{34}\text{O}_2$  Transfer 100 mL of isopropyl myristate into a centrifuge 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 myristate layer), and determine the pH of the residual water layer: not 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 parahydroxybenzoate**  $\text{C}_{10}\text{H}_{12}\text{O}_3$  Odorless and colorless fine crystals, or white, crystalline powder. Freely soluble in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

*Melting point* <2.60>: 84 – 86°C

*Residue on ignition* <2.44>: not more than 0.1%.

**Content**: not less than 99.0%. **Assay**—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS  
= 180.2 mg of  $\text{C}_{10}\text{H}_{12}\text{O}_3$

**4-Isopropylphenol**  $\text{C}_9\text{H}_{12}\text{O}$  White to reddish yellow, crystals or crystalline powder.

*Melting point* <2.60>: 59 – 63°C

**Isosorbide dinitrate for assay**  $\text{C}_6\text{H}_8\text{N}_2\text{O}_8$  [Same as the monograph Isosorbide Dinitrate. It contains not less than 99.0% of isosorbide dinitrate ( $\text{C}_6\text{H}_8\text{N}_2\text{O}_8$ ) meeting the following additional specifications.]

**Purity** Related substances—Dissolve 50 mg of isosorbide dinitrate for assay in 50 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of isosorbide dinitrate obtained from the sample solution is not larger than the peak area of isosorbide dinitrate from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating procedures in the Assay under Isosorbide Dinitrate Tablets.

**Time span of measurement**: About 2 times as long as the retention time of isosorbide dinitrate beginning after the solvent peak.

**System suitability**

**Test for required detectability**: Pipet 5 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 50 mL. Confirm that the peak area of isosorbide dinitrate obtained from 10  $\mu\text{L}$  of this solution is equivalent to 7 to 13% of that of isosorbide dinitrate from 10  $\mu\text{L}$  of the standard solution.

**System performance**: When the procedure is run with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 and not more than 1.5, respectively.

**System repeatability**: When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide dinitrate is not more than 2.0%.

**Isotonic sodium chloride solution** [Same as the name-sake monograph]

**Isoxsuprine hydrochloride for assay**  $\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{HCl}$   
[Same as the monograph Isoxsuprine Hydrochloride]

**Japanese acid clay** Natural hydrous aluminum silicate, grayish white powder, having a particle size of about 74  $\mu\text{m}$ .

*Loss on drying* <2.41>: not more than 10% (1 g, 105°C, 4 hours).

**Water adsorbing capacity**: not less than 2.5%. Weigh accurately about 10 g of Japanese acid clay in weighing bottle, allow to stand for 24 hours with cover in a chamber, in which humidity is maintained to 80% by means of sulfuric acid (specific gravity 1.19), reweigh, and determine the increase of mass of the sample.

**Jesaconitine for purity**  $\text{C}_{35}\text{H}_{49}\text{NO}_{12}$  A white powder.

Freely soluble in acetonitrile, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of jesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500  $\text{cm}^{-1}$ , 1715  $\text{cm}^{-1}$ , 1607  $\text{cm}^{-1}$ , 1281  $\text{cm}^{-1}$ , 1259  $\text{cm}^{-1}$ , 1099  $\text{cm}^{-1}$  and 772  $\text{cm}^{-1}$ .

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (258 nm): 270 – 291 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of jesaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of jesaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of jesaconitine and the solvent is not larger than the peak area of jesaconitine with the standard solution.

**Operating conditions**

**Detector, column, and column temperature:** Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

**Mobile phase:** A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

**Flow rate:** Adjust the flow rate so that the retention time of jesaconitine is about 36 minutes.

**Time span of measurement:** About 3 times as long as the retention time of jesaconitine.

**System suitability**

**Test for required detectability:** Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of jesaconitine obtained from 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that obtained from 10  $\mu\text{L}$  of the standard solution.

**System performance:** Dissolve 5 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 1 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu\text{L}$  of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of jesaconitine is not more than 1.5%.

**Water** <2.48>: not more than 1.0% [5 mg dried for not less

than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

**Josamycin**  $\text{C}_{42}\text{H}_{69}\text{NO}_{15}$  [Same as the namesake monograph]

**Josamycin propionate**  $\text{C}_{45}\text{H}_{73}\text{NO}_{16}$  [Same as the namesake monograph]

**Kainic acid** See kainic acid hydrate.

**Kainic acid for assay** See kainic acid hydrate.

**Kainic acid hydrate**  $\text{C}_{10}\text{H}_{15}\text{NO}_4 \cdot \text{H}_2\text{O}$  [Same as the namesake monograph]

**Kainic acid hydrate for assay** See kainic acid hydrate.

**Kanamycin sulfate**  $\text{C}_{18}\text{H}_{36}\text{N}_4\text{O}_{11} \cdot x\text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Karl Fischer TS** See Water Determination <2.48>.

**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** <2.56>: about 0.80

**Distilling range** <2.57>: 180 – 300°C

**Ketoconazole**  $\text{C}_{26}\text{H}_{28}\text{Cl}_2\text{N}_4\text{O}_4$  [Same as the namesake monograph]

**Ketoconazole for assay**  $\text{C}_{26}\text{H}_{28}\text{Cl}_2\text{N}_4\text{O}_4$  [Same as the monograph Ketoconazole. When dried, it contains not less than 99.5% of ketoconazole ( $\text{C}_{26}\text{H}_{28}\text{Cl}_2\text{N}_4\text{O}_4$ ).]

**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 solution. Perform the following tests with the sample solution: it meets the requirement of each test.

(i) Immediately after the 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. Proceed 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 solution at  $30 \pm 0.5^\circ\text{C}$  for 20 minutes, and proceed as directed in (i): kinin is not detected.

(iii) Perform the test with 0.5 mL 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 0.5 mL of 0.02 mol/L phosphate buffer solution, pH 8.0 containing 500  $\mu\text{g}$  of crystalline trypsin, previously warmed at  $30 \pm 0.5^\circ\text{C}$  for 5 minutes, warm this solution at  $30 \pm 0.5^\circ\text{C}$  for 5 minutes, add 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Then boil for 3 minutes, 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 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution add trichloroacetic acid-gelatin-tris buffer solution to

make 20 mL, then proceed as directed in (i), and determine the amount,  $B_K$ , of kinin per well. Calculate the kinin-releasing activity per mg by the following equation: not less than 10  $\mu\text{g}$  bradykinin equivalent per mg.

$$\text{Kinin-releasing activity per mg } (\mu\text{g bradykinin equivalent/mg}) = B_K \times 0.0096$$

**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 each mL to release kinin corresponding to not less than 1  $\mu\text{g}$  of bradykinin.

**Labetalol hydrochloride**  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$  [Same as the namesake monograph]

**Labetalol hydrochloride for assay**  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$  [Same as the monograph Labetalol Hydrochloride. However, when dried, it contains not less than 99.0% of labetalol hydrochloride ( $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$ ).]

**Lactic acid**  $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$  [K 8726, Special class]

**Lactic acid TS** Dissolve 12.0 g of lactic acid in water to make 100 mL.

**$\alpha$ -Lactalbumin** White powder. Derived from milk. Molecular weight of about 14,200.

**Lactobionic acid**  $\text{C}_{12}\text{H}_{22}\text{O}_{12}$  Colorless crystals or white crystalline powder, having no odor.

*Melting point* <2.60>: 113 – 118°C

*Purity*—Dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

**$\beta$ -Lactoglobulin** Prepare from milk. White to light yellow powder.

*Nitrogen content* <1.08>: not less than 14% (calculated on the dried basis).

**Lactose** See lactose monohydrate.

**$\alpha$ -Lactose and  $\beta$ -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 12 mL of bromothymol blue-sodium hydroxide TS to 1000 mL of the medium. Then dispense portions of about 10 mL 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 not 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 330 mL 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 portions 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 monohydrate**  $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_2\text{O}$  [Same as the monograph Lactose].

**Lactose substrate TS** Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

**Lactose substrate TS for  $\beta$ -galactosidase (penicillium)** Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

**Lanthanum-alizarin complexone TS** To 1 mL of ammonia water (28) add 10 mL of water. To 4 mL of this solution add 4 mL of a solution of ammonium acetate (1 in 5) and 192 mg of alizarin complexone, and label this solution as alizarin complexone stock solution. Dissolve 41 g of sodium acetate trihydrate in 400 mL of water, and add 24 mL of acetic acid (100). To this solution add the total volume of the alizarin complexone stock solution, add 400 mL of acetone, and label this solution as alizarin complexone solution. To 10 mL of diluted hydrochloric acid (1 in 6) add 163 mg of lanthanum (III) oxide, heat to dissolve, and label this solution as lanthanum solution. To the alizarin complexone solution add the lanthanum solution, and mix. After cooling, adjust to pH 4.7 with acetic acid (100) or ammonia water (28), and add water to make 1000 mL. Prepare before use.

**Lanthanum chloride TS** To 58.65 g of lanthanum (III) oxide add 100 mL of hydrochloric acid, and boil. After cooling, add water to make 1000 mL.

**Lanthanum (III) oxide**  $\text{La}_2\text{O}_3$  White crystals.  
*Loss on ignition* <2.43>: not more than 0.5% (1 g, 1000°C, 1 hour)

**Lauromacrogol** [Same as the namesake monograph]

**Lead acetate** See lead (II) acetate trihydrate.

**Lead acetate TS** See lead (II) acetate TS.

**Lead monoxide** See lead (II) oxide.

**Lead nitrate** See lead (II) nitrate.

**Lead dioxide** See lead (IV) oxide.

**Lead subacetate TS** Place the yellowish mixture, obtained by triturating 3 g of lead (II) acetate trihydrate and 1 g of lead (II) oxide with 0.5 mL of water, in a beaker, and heat on a water bath, covering with a watch glass, until it shows a homogeneous, white to reddish white color. Then add 9.5 mL of hot water in small portions, cover it again with a watch glass, and set it aside. Decant the supernatant liquid, and adjust the specific gravity to 1.23 to 1.24 (15°C) by adding water. Preserve in tightly stoppered bottles.

**Lead subacetate TS, dilute** To 2 mL of lead subacetate TS add freshly boiled and cooled water to make 100 mL. Prepare before use.

**Lead (II) acetate trihydrate**  $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$  [K 8374, Special class]

**Lead (II) acetate TS** To 9.5 g of lead (II) acetate trihydrate add freshly boiled and cooled water to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/L).

**Lead (II) nitrate**  $\text{Pb}(\text{NO}_3)_2$  [K 8563, Special class]

**Lead (II) oxide** PbO [K 8090, Special class]

**Lead (IV) oxide** PbO<sub>2</sub> 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 <1.09> (3) for lead salt.

**L-Leucine** C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub> [Same as the namesake monograph]

**L-Leucine for assay** C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub> [Same as the monograph L-Leucine. When dried, it contains not less than 99.0% of L-leucine (C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub>).]

**Levallorphan tartrate for assay** C<sub>19</sub>H<sub>25</sub>NO·C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> [Same as the monograph Levallorphan Tartrate. When dried, it contains not less than 99.0% of C<sub>19</sub>H<sub>25</sub>NO·C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>.]

**Levothyroxine sodium** See levothyroxine sodium hydrate.

**Levothyroxine sodium for thin-layer chromatography** See levothyroxine sodium hydrate for thin-layer chromatography.

**Levothyroxine sodium hydrate** C<sub>15</sub>H<sub>10</sub>I<sub>4</sub>NNaO<sub>4</sub>·xH<sub>2</sub>O [Same as the namesake monograph]

**Levothyroxine sodium hydrate for thin-layer chromatography** C<sub>15</sub>H<sub>10</sub>I<sub>4</sub>NNaO<sub>4</sub>·xH<sub>2</sub>O [Same as the monograph Levothyroxine Sodium Hydrate. Proceed the test as directed in the Identification (3) under Levothyroxine Sodium Hydrate: any spot other than the principal spot at the Rf value of about 0.26 does not appear.]

**Lidocaine for assay** (C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O) [same as the monograph Lidocaine]

**Limonene** C<sub>10</sub>H<sub>16</sub> Clear and colorless liquid, having a specific perfume and a bitter taste.

*Refractive index* <2.45>  $n_D^{20}$ : 1.427 – 1.474

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.841 – 0.846

*Melting point* <2.60>: 176 – 177°C

**Purity** Related substances—Dissolve 0.1 g of limonene in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <2.02> 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 beginning after the solvent peak.

**Limonin for thin-layer chromatography** C<sub>26</sub>H<sub>30</sub>O<sub>8</sub> White crystals or crystalline powder. Slightly soluble in methanol and in ethyl acetate, and practically insoluble in water and in ethanol (99.5). *Melting point:* about 290°C.

**Identification** Determine the infrared absorption spectrum as directed in the potassium bromide disk method

under Infrared-visible Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1759 cm<sup>-1</sup>, 1709 cm<sup>-1</sup>, 1166 cm<sup>-1</sup>, 798 cm<sup>-1</sup> and 601 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 1 mg of limonin for thin-layer chromatography in 1 mL of ethyl acetate, and perform the test with 1 μL of this solution as directed in the Identification (2) under Oregedokuto Extract: no spot other than the principal spot (Rf value is about 0.4) appears.

**(Z)-Ligustilide for thin-layer chromatography** C<sub>12</sub>H<sub>14</sub>O<sub>2</sub> A clear, yellow-grown liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the absorption spectrum of a solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 320 nm and 324 nm.

**Purity** Related substances—Dissolve 1 mg in 10 mL of methanol. Proceed the test with 1 μL of this solution as directed in the Identification (5) under Hochuekkito Extract: no spot other than the principle spot of around Rf 0.6 appears.

**Liothyronine sodium** C<sub>15</sub>H<sub>11</sub>I<sub>3</sub>NNaO<sub>4</sub> [Same as the namesake monograph]

**Liothyronine sodium for thin-layer chromatography** [Same as the monograph Liothyronine Sodium. Proceed as directed for the Identification (1) under Liothyronine Sodium Tablets: any spot other than the principal spot at the Rf value of 0.3 to 0.4 does not appear.]

**Liquid paraffin** See paraffin, liquid.

**Liquiritin for thin-layer chromatography** C<sub>21</sub>H<sub>22</sub>O<sub>9</sub>·xH<sub>2</sub>O White crystals or crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 210°C (with decomposition).

**Identification**—Determine the absorption spectrum of a solution in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, and between 275 nm and 279 nm.

**Purity** Related substances—Dissolve 1.0 mg in 1 mL of methanol, and perform the test with 1 μL of this solution as directed in the Identification (5) under Kakkonto Extract: no spot other than the principal spot (Rf value is about 0.4) appears.

**Lisinopril** See lisinopril hydrate.

**Lisinopril for assay** See lisinopril hydrate for assay.

**Lisinopril hydrate** C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub>·2H<sub>2</sub>O [Same as the namesake monograph]

**Lisinopril hydrate for assay** C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub>·2H<sub>2</sub>O [Same as the monograph Lisinopril Hydrate. It contains not less than 99.5% of lisinopril (C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub>: 405.49), calculated on the anhydrous basis.]

**Lithium acetate dihydrate** CH<sub>3</sub>COOLi·2H<sub>2</sub>O Colorless crystals.

Dilute acetic acid insoluble substances—To 40.0 g of lithium acetate dihydrate add 45 mL 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 dihydrate, add exactly 50 mL of acetic acid (100) and exactly 5 mL of acetic anhydride, dissolve by heating in a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS after cooling (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 10.20 mg of CH<sub>3</sub>COOLi·2H<sub>2</sub>O

**Lithium bromide** LiBr White crystals or crystalline powder. It is hygroscopic.

**Purity** (1) Chloride <1.03>: not more than 0.1%.

(2) Sulfate <1.14>: not more than 0.01%.

**Lithium chloride** LiCl White crystals or masses.

**Identification**—Perform the test as directed under Flame Coloration Test (1) <1.04>: a persistent red color appears.

**Lithium perchlorate** LiClO<sub>4</sub> White, crystals or crystalline powder.

**Content:** not less than 98%. **Assay**—Accurately weigh about 0.2 g of lithium perchlorate, dissolve in 30 mL of water. Transfer the solution to a chromatographic column, prepared by pouring about 25 mL of strongly acidic ion-exchange resin (H type) for column chromatography into a chromatographic tube about 11 mm in inside diameter and about 300 mm in height (after adding 200 mL of 1 mol/L hydrochloric acid TS and flowing at a flow rate of 3–4 mL per minute, wash the chromatographic column with water until the color of the rinse water changes to yellowish red when adding methyl orange TS to the eluate), and flow at a flow rate of 3–4 mL per minute. Then, wash the column with about 30 mL of water at a flow rate of 3–4 mL per minute 5 times. Combine the rinse water and the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 10.64 mg of LiClO<sub>4</sub>

**Lithium sulfate** See lithium sulfate monohydrate.

**Lithium sulfate monohydrate** Li<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O [K 8994, Special class]

#### Lithocholic acid for thin-layer chromatography

C<sub>24</sub>H<sub>40</sub>O<sub>3</sub> White crystals or crystalline powder. Soluble in ethanol (95), in acetic acid (100) and in acetone, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 186°C.

**Purity** Related substances—Dissolve 25 mg of lithocholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 25 mL. Dilute 1.0 mL of this solution with a mixture of chloroform and ethanol (95) (9:1) to make exactly 100 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 with the R<sub>f</sub> value of about 0.7 does not appear.

**Content:** 98.0%. **Assay**—Weigh accurately about 0.5 g of lithocholic acid for thin-layer chromatography, previously dried at 80°C for 4 hours under reduced pressure (phospho-

rus (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, titrate <2.50> with 0.1 mol/L sodium hydroxide VS, add 100 mL of freshly boiled and cooled water near the end point, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 37.66 mg of C<sub>24</sub>H<sub>40</sub>O<sub>3</sub>

#### Locke-Ringer's TS

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride dihydrate	0.24 g
Magnesium chloride hexahydrate	0.2 g
Sodium hydrogen carbonate	0.5 g
Dextrose	0.5 g
Water, freshly distilled with a hard-glass apparatus	a sufficient quantity
To make	1000 mL

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** Loganin for thin-layer chromatography meeting the following additional specifications.

**Absorbance** <2.24> E<sub>1%<sup>1</sup>cm</sub> (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 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

Time span of measurement: About 3 times as long as the retention time of loganin.

**System suitability**

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of loganin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of loganin from 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

**Loganin for component determination** See loganin for assay.

**Loganin for thin-layer chromatography** C<sub>17</sub>H<sub>26</sub>O<sub>10</sub>

White, crystals or crystalline powder. Soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5). Melting point: 221–227°C.

**Purity** Related substances—Dissolve 1.0 mg of loganin for thin-layer chromatography in 2 mL of methanol.

Perform the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Cornus Fruit: any spot other than the principal spot at the  $R_f$  value of about 0.4 does not appear.

**Lovastatin**  $\text{C}_{24}\text{H}_{36}\text{O}_5$  White crystals or crystalline powder. Soluble in acetonitrile and in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

*Optical rotation* <2.49>  $[\alpha]_{\text{D}}^{20}$ : +325 – +340° (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

*Loss on drying* <2.41>: Not more than 1.0% (1 g, under reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Low-molecular weight heparin for calculation of molecular mass.**

It is a low-molecular weight heparin with a disaccharide unit prepared, and display the molecular mass distribution between 600 and more than 10,000. When the average of molecular mass of Low-molecular weight heparin international standard is determined as a reference with this, the difference compared as a reference with the Low-molecular weight heparin international standard is not less than 5%.

**Luteolin for thin-layer chromatography**  $\text{C}_{15}\text{H}_{10}\text{O}_6$  Light yellow to yellow-brown crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 310°C (with decomposition).

*Purity* Related substances—Dissolve 1.0 mg of luteolin for thin-layer chromatography in 1 mL of methanol. Proceed the test with 10  $\mu\text{L}$  of this solution as directed in the Identification under Chrysanthemum Flower: any spot other than the principal spot of  $R_f$  about 0.7 does not appear.

**Lysate reagent** A lyophilized product obtained from amebocyte lysate of horseshoe crab (*Limulus polyphemus* or *Tachyplesus tridentatus*). Amebocyte lysate preparations which do not react to  $\beta$ -glucans are available: they are prepared by removing the G factor reacting to  $\beta$ -glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate.

**Lysate TS** Dissolve a lysate reagent in water for bacterial endotoxins test, or in a suitable buffer, by gentle stirring.

**Lysil endopeptidase** White powder or masses, An exotoxin produced by *Achromobacter*. Molecular weight: 27,500.

**L-Lysine hydrochloride**  $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2 \cdot \text{HCl}$  [Same as the namesake monograph]

**Macrogol 600**  $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$ ,  $n = 11 - 13$  Clear, colorless, viscous liquid or a white, petrolatum-like solid, having a faint, characteristic odor. Very soluble in water, in ethanol (95), in acetone and in macrogol 400, soluble in diethyl ether, and practically insoluble in petroleum benzene. Congealing point: 18 – 23°C

*Average molecular weight*: When perform the test as directed in the Average molecular weight test under Macrogol 400, it is between 570 and 630.

**4-(N-Maleimidylmethyl)-cyclohexane-1-carboxylate-N-hydroxysuccinimide ester**  $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_6$  Colorless crystals. Being decomposed by acid and alkaline treatment.

**Magnesia TS** Dissolve 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride in 65 mL of water,

add 35 mL 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 [K 8875, Special class]

**Magnesium chloride** See magnesium chloride hexahydrate.

**Magnesium chloride hexahydrate**  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  [K 8159, Special class]

**Magnesium nitrate** See magnesium nitrate hexahydrate.

**Magnesium nitrate hexahydrate**  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  [K 8567, Special class]

**Magnesium oxide** MgO [K 8432, Special class]

**Magnesium powder** Mg [K 8876, Special class]

**Magnesium sulfate** See magnesium sulfate heptahydrate.

**Magnesium sulfate heptahydrate**  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  [K 8995, Special class]

**Magnesium sulfate TS** Dissolve 12 g of magnesium sulfate hexahydrate in water to make 100 mL (0.5 mol/L).

**Magnolol for assay** Use magnolol for thin-layer chromatography meeting the following additional specifications.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (290 nm): 270 – 293 (10 mg, methanol, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the sample.

*Purity* Related substances—Dissolve 5.0 mg of magnolol for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak from these solutions by the automatic integration method: the total area of peaks other than the peak of magnolol from the sample solution is not larger than the peak area of magnolol from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Magnolia Bark.

Time span of measurement: About 3 times as long as the retention time of magnolol.

System suitability

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay under Magnolia Bark.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of magnolol obtained with 10  $\mu\text{L}$  of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu\text{L}$  of the standard solution.

**Magnolol for component determination** See magnolol for assay.

**Magnolol for thin-layer chromatography**  $\text{C}_{18}\text{H}_{18}\text{O}_2$  Odorless, white crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble

in water. Melting point: about 102°C.

**Identification**—Determine the absorption spectrum of a solution of magnolol for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 287 nm and 291 nm.

**Purity** Related substances—Dissolve 1.0 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, acetone and acetic acid (100) (20:15:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of around *Rf* value of 0.5 does not appear.

**Malachite green** See malachite green oxalate.

**Malachite green oxalate**  $\text{C}_{52}\text{H}_{54}\text{N}_2\text{O}_{12}$  [K 8878, Malachite green (oxalate), Special class]

**Maleic acid**  $\text{C}_4\text{H}_4\text{O}_4$  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 <2.25>: it exhibits absorption at the wave numbers of about 1706  $\text{cm}^{-1}$ , 1637  $\text{cm}^{-1}$ , 1587  $\text{cm}^{-1}$ , 1567  $\text{cm}^{-1}$ , 1436  $\text{cm}^{-1}$ , 1263  $\text{cm}^{-1}$ , 876  $\text{cm}^{-1}$  and 786  $\text{cm}^{-1}$ .

**4-(Maleimidomethyl)cyclohexylcarbonic acid-*N*-hydroxy-succinimide ester**  $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2$  Colorless crystals, which is decomposed by acid or alkali.

**Maltose** See maltose monohydrate.

**Maltose monohydrate**  $\text{C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}$  [Same as the namesake monograph].

**Maltotriose**  $\text{C}_{18}\text{H}_{32}\text{O}_{16}$  A white powder.

**Identification**—Determine the infrared absorption spectrum of maltotriose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3420  $\text{cm}^{-1}$ , 1420  $\text{cm}^{-1}$ , 1153  $\text{cm}^{-1}$  and 1024  $\text{cm}^{-1}$ .

**Manganese dioxide**  $\text{MnO}_2$  Black to black-brown, masses or powder.

**Identification**—To 0.5 g add 20 mL of water and 3 mL of hydrochloric acid, and 3 mL of hydrogen peroxide (30). Alkalinize the solution with ammonia solution (28) while cooling, and add 25 mL of hydrogen sulfide TS: pale red precipitates appear.

**Manganese (II) nitrate hexahydrate**  $\text{Mn}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$  [K 8568, Special class]

**D-Mannitol**  $\text{C}_6\text{H}_{14}\text{O}_6$  [Same as the monograph D-Mannitol]

**D-Mannosamine hydrochloride**  $\text{C}_6\text{H}_{13}\text{NO}_5\cdot\text{HCl}$  White powder. Melting point: about 168°C (with decomposition).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $-4.2 - -3.2^\circ$  (0.4 g, water, 20 mL, 100 mm).

**D-Mannose**  $\text{C}_6\text{H}_{12}\text{O}_6$  White crystal or crystalline powder. It is very soluble in water. Melting point: about 132°C (with decomposition).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $+13.7 - +14.7^\circ$  (4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm).

**Marker protein for celmoleukin molecular weight determination** Add 10  $\mu\text{L}$  of cytochrome C prepared to a concentration of 2 mg per mL to 10  $\mu\text{L}$  of a commercially available marker protein with a known molecular weight (6 ingredients: phosphorylase b, bovine serum albumin, ovalbumin, carbonic dehydratase, soy trypsin inhibitor, and lysozyme) and then dilute 10-fold with buffer solution for celmoleukin.

**Identification**—Use the solution to be examined as the sample solution. Separately, to an amount of cytochrom C add distilled water for injection so that each mL contains 100 mg of protein, and use this as the standard solution. When 20  $\mu\text{L}$  each of the sample solution and standard solution are tested using SDS polyacrylamide gel electrophoresis under the operating conditions outlined in the Identification (3) of Celmoleukin (Genetical Recombination), the sample solution exhibits 7 major electrophoretic bands. Furthermore, the degree of mobility of the sample solution cytochrome C is consistent with that of the band obtained from the standard solution.

**Meat extract** Concentrated extract of fresh meat of bovine, equine or other animals. A yellow-brown to dark brown paste-like mass, having a meat-like odor.

**Mebendazole**  $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_3$  White powder. Practically insoluble in water and in ethanol (95).

**Medium for float culture** Dissolve 6.000 g of sodium chloride, 0.400 g of potassium chloride, 0.677 g of anhydrous sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), 0.100 g of calcium nitrite tetrahydrate, 0.100 g of magnesium sulfate hydrate, 2.000 g of glucose, 0.164 g of sodium succinate hepta-hydrate, 46 mg of succinic acid, 0.240 g of L-arginine hydrochloride, 56.8 mg of L-asparagine monohydrate, 20 mg of L-aspartic acid, 72.9 mg of L-cysteine hydrochloride monohydrate, 20 mg of L-glutamic acid, 1 mg of glutathione, 10 mg of glycine, 20.3 mg of L-histidine hydrochloride monohydrate, 20 mg of L-hydroxyproline, 50 mg of L-isoleucine, 40 mg of L-lysine hydrochloride, 15 mg of methionine, 20 mg of L-threonine, 5 mg of L-tryptophan, 20 mg of L-valine, 50 mg of L-leucine, 15 mg of L-phenylalanine, 20 mg of L-proline, 30 mg of L-serine, 20 mg of L-tyrosine, 0.2 mg of D-biotin (crystals), 0.25 mg of calcium pantothenate, 3 mg of choline chloride, 35 mg of *i*-inositol, 1 mg of 4-aminobenzoic acid, 5  $\mu\text{g}$  of cyanocobalamin, 1 mg of folic acid, 1 mg of nicotinamide, 0.2 mg of riboflavin, 1 mg of thiamine hydrochloride, 1 mg of pyridoxine hydrochloride, and 5 mg of phenol red in a suitable amount of water, add 1 mL of kanamycin sulfate solution (3 in 50), add water to make 1000 mL, and then sterilize by autoclaving for 15 minutes at 121°C. After cooling, add 10 mL of L-glutamine solution (3 in 100) and 20 mL of 7% sodium bicarbonate injection, and then mix. Store at 4°C.

**Mefloquin hydrochloride** [Same as the namesake monograph]

**Mefruside for assay**  $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_5\text{S}_2$  [Same as the monograph Mefruside. When dried, it contains not less than 99.0% of mefruside ( $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_5\text{S}_2$ ).]

**Meglumine**  $\text{C}_7\text{H}_{17}\text{NO}_5$  [same as the namesake mono-

graph]

**Mentha herb** [Same as the namesake monograph]

**Mentha oil** [Same as the namesake monograph]

**Menthol**  $C_{10}H_{20}O$  [Same as the monograph *dl*-Menthol or *l*-Menthol]

***l*-Menthol for assay** [Same as the monograph *l*-Menthol. It contains not less than 99.0% of  $C_{10}H_{20}O$  and meets the following additional specifications.]

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $-48.0 - -51.0^\circ$  (2.5 g, ethanol (95), 25 mL, 100 mm).

**Purity** Related substances—Dissolve 0.10 g of *l*-menthol for assay in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5  $\mu$ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, measure each peak area of these solutions by the automatic integration method: the total peak area other than the peak area of *l*-menthol from the sample solution is not larger than the peak area of *l*-menthol from the standard solution (1).

**Operating conditions**

Proceed the operating conditions in the Assay under *Mentha 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 *l*-menthol obtained from 5  $\mu$ L of the standard solution (2) can be measured, and the peak height of *l*-menthol from 5  $\mu$ L of the standard solution (1) is about 20% of the full scale.

**Time span of measurement:** About twice as long as the retention time of *l*-menthol beginning after the solvent peak.

**Mepivacaine hydrochloride for assay**  $C_{15}H_{22}N_2O.HCl$  [Same as the monograph Mepivacaine Hydrochloride. When dried, it contains not less than 99.0% of mepivacaine hydrochloride ( $C_{15}H_{22}N_2O.HCl$ ).]

**Mercapto acetic acid**  $HSCH_2COOH$  [K 8630, Special class] Place in an ampule, and preserve in a dark, cold place. Do not use after storing for a long period.

**Mercaptoethanesulfonic acid**  $C_2H_6O_3S_2$  Prepared for amino acid analysis or biochemistry.

**2-Mercaptoethanol**  $HSCH_2CH_2OH$  Clear and colorless liquid.

**Specific gravity** <2.56>  $d_4^{20}$ : 1.112 - 1.117

**Content:** not less than 97.0%. **Assay**—Perform the test with 0.6  $\mu$ L of the substance to be examined as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of each component by the automatic integration method.

**Content (%)** = (the peak area of 2-mercaptoethanol/the total of the peak areas of each component)  $\times$  100

**Operating conditions**

**Detector:** A hydrogen flame-ionization detector.

**Column:** A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (177–250  $\mu$ m in particle diameter) coated in 20% with

50% phenyl-methyl silicone polymer for gas chromatography.

**Column temperature:** A constant temperature of about 120°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust the flow rate of about 50 mL/min and so that the retention time of 2-mercaptoethanol is 3–4 minutes.

**Time span of measurement:** About 7 times as long as the retention time of 2-mercaptoethanol.

**Mercaptopurine** See mercaptopurine hydrate.

**Mercaptopurine hydrate**  $C_5H_4N_4S.H_2O$  [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 chloride** See mercury (II) chloride.

**Mercury** Hg [K 8572, Special class]

**Mercury (II) acetate**  $Hg(CH_3COO)_2$  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 20 mL of water, and use this as the sample solution. To 10 mL 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 acetic acid (100) for nonaqueous titration to make 100 mL.

**Mercury (II) chloride**  $HgCl_2$  [K 8139, Special class]

**Mercury (II) chloride TS** Dissolve 5.4 g of mercury (II) chloride in water to make 100 mL.

**Mesaconitine for purity**  $C_{33}H_{45}NO_{11}$  White, crystals or crystalline powder. Slightly soluble in acetonitrile and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 190°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of mesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3510  $cm^{-1}$ , 1713  $cm^{-1}$ , 1277  $cm^{-1}$ , 1116  $cm^{-1}$ , 1098  $cm^{-1}$  and 717  $cm^{-1}$ .

**Absorbance** <2.24>  $E_{1\%}^{1\text{cm}}$  (230 nm): 211–247 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

**Purity** Related substances—(1) Dissolve 5.0 mg of mesaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test

as directed in the Identification under Processed Aconite Root: the spot other than the principal spot is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of mesaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of mesaconitine and the solvent is not larger than the peak area of mesaconitine with the standard solution.

#### Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of mesaconitine.

#### System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of mesaconitine obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10  $\mu$ L of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10  $\mu$ L of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mesaconitine is not more than 1.5%.

*Water* <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

**Mesityl oxide**  $\text{CH}_3\text{COCH}=\text{C}(\text{CH}_3)_2$  A colorless or pale yellow, clear liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.850 – 0.860

**Metacresol purple**  $\text{CH}_3\text{C}_6\text{H}_4(\text{OH})$  [K 8889, Special class]

**Metacresol purple TS** Dissolve 0.10 g of metacresol purple in 13 mL of 0.01 mol/L sodium hydroxide TS, and add water to make 100 mL.

**Metacycline hydrochloride**  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_8 \cdot \text{HCl}$  Yellow to dark yellow, crystals or crystalline powder.

*Purity* Related substances—Dissolve 20 mg of metacycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20  $\mu$ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate,

determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of peaks other than metacycline is not more than 10%.

**Metallic sodium** See sodium.

**Metanil yellow**  $\text{C}_{18}\text{H}_{14}\text{N}_3\text{NaO}_3\text{S}$  Yellow-brown powder. Sparingly soluble in water, and very slightly soluble in ethanol (95) and in *N,N*-dimethylformamide.

**Metanil yellow TS** Dissolve 0.1 g of metanil yellow in 200 mL of *N,N*-dimethylformamide.

**Metaphosphoric acid**  $\text{HPO}_3$  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.

**Metenolone enanthate**  $\text{C}_{27}\text{H}_{42}\text{O}_3$  [Same as the name-sake monograph]

**Metenolone enanthate for assay**  $\text{C}_{27}\text{H}_{42}\text{O}_3$  To 1 g of metenolone enanthate add 30 mL of water, and add slowly 70 mL of methanol with warming to dissolve. Filter while hot, and allow the filtrate to stand on a water bath for 30 minutes. Allow to stand overnight in a cold place, collect the crystals thus formed, and wash with a small amount of diluted methanol (1 in 3). Recrystallize in the same manner, and dry the crystals in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (242 nm): 321 – 328 (1 mg, methanol, 100 mL).

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +40 – +42° (0.2 g, chloroform, 10 mL, 100 mm).

*Melting point* <2.60>: 69 – 72°C

*Purity* Related substances—Dissolve 50 mg of metenolone enanthate for assay in chloroform to make exactly 10 mL, and use this solution as the sample solution. Proceed with 10  $\mu$ L of this solution as directed in the Purity (3) under Metenolone Enanthate: any spot other than the principal spot does not appear.

**Metformin hydrochloride for assay**  $\text{C}_4\text{H}_{11}\text{N}_5 \cdot \text{HCl}$  [Same as the monograph Metformin Hydrochloride. When dried, it contains not less than 99.0% of metformin hydrochloride ( $\text{C}_4\text{H}_{11}\text{N}_5 \cdot \text{HCl}$ ).]

**Methanesulfonic acid**  $\text{CH}_3\text{SO}_3\text{H}$  Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether.

*Congealing point* <2.42>: 15 – 20°C

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.483 – 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 <2.50> with 1 mol/L sodium hydroxide VS

(indicator: 2 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 96.11 mg of  $\text{CH}_3\text{SO}_3\text{H}$

**Methanesulfonic acid TS** To 35 mL of methanesulfonic acid add 20 mL of acetic acid (100) and water to make 500 mL.

**0.1 mol/L Methanesulfonic acid TS** To 4.8 g of methanesulfonic acid add water to make 500 mL.

**Methanol**  $\text{CH}_3\text{OH}$  [K 8891, Special class]

**Methanol, anhydrous**  $\text{CH}_4\text{O}$  To 1000 mL of methanol add 5 g of magnesium powder. If necessary, add 0.1 mL of mercury (II) chloride TS to start the reaction. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate protecting from moisture. Water content per mL is not more than 0.3 mg.

**Methanol for Karl Fischer method** See Water Determination <2.48>.

**Methanol for liquid chromatography**  $\text{CH}_3\text{OH}$  A clear, colorless liquid. Mixable with water.

**Purity** Ultraviolet-absorbing substances—Perform the test as directed in Ultraviolet-visible Spectrophotometry <2.24> using water as the blank: the absorbances at 210 nm, at 220 nm, at 230 nm, at 240 nm and at 254 nm are not more than 0.70, 0.30, 0.15, 0.07 and 0.02, respectively.

**Methanol-free ethanol** See ethanol (95), methanol-free.

**Methanol-free ethanol (95)** See ethanol (95), methanol-free.

**Methanol, purified** Distil methanol before use.

**Methionin** See L-methionine.

**L-Methionine**  $\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$  [Same as the namesake monograph]

**Methotrexate**  $\text{C}_{20}\text{H}_{22}\text{N}_8\text{O}_5$  [Same as the namesake monograph]

**4-Methoxybenzaldehyde**  $\text{C}_8\text{H}_8\text{O}_2$  Clear, colorless to light yellow liquid. Miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

**Specific gravity** <2.56>  $d_4^{20}$ : 1.123 – 1.129

**Content:** not less than 97.0%. **Assay**—Weigh accurately about 0.8 g of 4-methoxybenzaldehyde, add exactly 7.5 mL of hydroxylamine TS, shake well, allow to stand for 30 minutes, and titrate <2.50> with 0.5 mol/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.

Each mL of 0.5 mol/L hydrochloric acid VS  
= 68.08 mg of  $\text{C}_8\text{H}_8\text{O}_2$

**4-Methoxybenzaldehyde-acetic acid TS** To 0.5 mL of 4-methoxybenzaldehyde add acetic acid (100) to make 100 mL.

**4-Methoxybenzaldehyde-sulfuric acid TS** To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix thoroughly.

**4-Methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray** To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde, mix gently, add gently 0.5 mL of sul-

furic acid and 0.1 mL of acetic acid (100) in this order, and mix well.

**(E)-2-Methoxycinnamaldehyde for thin-layer chromatography**  $\text{C}_{10}\text{H}_{10}\text{O}_2$  White to yellow, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 44 – 50°C.

**Identification**—(1) Determine the absorption spectrum of a solution of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 282 nm and 286 nm, and between 331 nm and 335 nm.

(2) Determine the infrared absorption spectrum of (E)-2-methoxycinnamaldehyde for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1675  $\text{cm}^{-1}$ , 1620  $\text{cm}^{-1}$ , 1490  $\text{cm}^{-1}$ , 1470  $\text{cm}^{-1}$ , 1295  $\text{cm}^{-1}$ , 1165  $\text{cm}^{-1}$ , 1130  $\text{cm}^{-1}$ , 1025  $\text{cm}^{-1}$  and 600  $\text{cm}^{-1}$ .

**Purity** Related substances—Dissolve 10 mg of (E)-2-methoxycinnamaldehyde for thin-layer chromatography in 5 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with 5  $\mu\text{L}$  each of the sample solution and standard solution as directed in the Identification (5) (ii) under Goshajinkigan Extract: the spots other than the principal spot appeared at about  $R_f$  0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

**2-Methoxyethanol**  $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}$  [K 8895, Special class]

**2-Methoxy-4-methylphenol**  $\text{C}_8\text{H}_{10}\text{O}_2$  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 <2.25>: it exhibits absorption at the wave numbers of about 1511  $\text{cm}^{-1}$ , 1423  $\text{cm}^{-1}$ , 1361  $\text{cm}^{-1}$ , 1268  $\text{cm}^{-1}$ , 1231  $\text{cm}^{-1}$ , 1202  $\text{cm}^{-1}$ , 1148  $\text{cm}^{-1}$ , 1120  $\text{cm}^{-1}$ , 1031  $\text{cm}^{-1}$ , 919  $\text{cm}^{-1}$ , 807  $\text{cm}^{-1}$  and 788  $\text{cm}^{-1}$ .

**Purity** Related substances—Perform the test with 0.2  $\mu\text{L}$  of 2-methoxy-4-methylphenol as directed under Gas Chromatography <2.02> 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  $\mu\text{m}$  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 100 mL, and use this solution as the solution for system suitability test. Proceed with 1  $\mu$ L 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  $\mu$ L 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 5 mL of 1-methoxy-2-propanol add 20 mL of water, and mix: the solution is clear.

*Refractive index* <2.45>  $n_D^{20}$ : 1.402 – 1.405

*Specific gravity* <2.56>  $d_4^{20}$ : 0.920 – 0.925

*Water* <2.48>: not more than 0.5% (5 g).

*Content*: not less than 98.0%. *Assay*—Proceed as directed under Gas Chromatography <2.02> using the area percentage method according to the following conditions:

Operating conditions

Detector: Thermal conductivity 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  $\mu$ m) 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: A constant flow rate of 20 mL per minute.

**Methyl acetate**  $CH_3COOCH_3$  [K 8382, Special class]

**p-Methyl aminophenol sulfate** See 4-methyl aminophenol sulfate.

**4-Methyl aminophenol sulfate**  $(HOC_6H_4NHCH_3)_2 \cdot H_2SO_4$  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 aminophenol 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* <2.56>  $d_4^{20}$ : 1.050 – 1.065

*Boiling point* <2.57>: 200 – 202°C

*Water* <2.48>: less than 0.1%.

**2-Methylamino pyridine for Karl Fischer method** See Water Determination <2.48>.

**Methyl behenate**  $C_{23}H_{46}O_2$  White, odorless and tasteless, scaly crystals or powder. Dissolves in acetone, in diethyl ether and in chloroform.

*Melting point* <2.60>: 54°C

*Saponification value* <1.13>: 155.5 – 158.5

**Methyl benzoate**  $C_6H_5COOCH_3$  Clear, colorless liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.515 – 1.520

*Specific gravity* <2.56>  $d_4^{20}$ : 1.087 – 1.095

*Purity*—Dissolve 0.1 mL of methyl benzoate in the mobile phase in Assay under Thiamine Hydrochloride to make 50 mL. Perform the test as directed under Liquid Chromatography <2.01> with 10  $\mu$ L of this solution according to the Assay under Thiamine Hydrochloride. Measure each peak area by the automatic integration method in a range about twice the retention time of methyl benzoate, and calculate the amount of methyl benzoate by the area percentage method: it shows the purity of not less than 99.0%.

**Methyl benzoate for estriol test**  $C_8H_8O_2$  Clear, colorless liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.515 – 1.520

*Specific gravity* <2.56>  $d_4^{20}$ : 1.087 – 1.095

*Acid value* <1.13>: not more than 0.5.

**D-(+)- $\alpha$ -Methylbenzylamine**  $C_6H_5CH(CH_3)NH_2$  Colorless or pale yellow clear liquid, having an amine like odor. Miscible with ethanol (95) and with acetone, and slightly soluble in water.

*Refractive index* <2.45>  $n_D^{20}$ : 1.524–1.529

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +37 – +41° (50 mm).

*Content*: not less than 98.0%. *Assay*—Perform the test with exact 0.6  $\mu$ L of D-(+)- $\alpha$ -methylbenzylamine as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of D-(+)- $\alpha$ -methylbenzylamine.

Operating conditions

Detector: 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 (180 to 250  $\mu$ m in particle diameter) coated with polyethylene glycol 20 M for gas chromatography and potassium hydroxide at the ratio of 10% and 5%, respectively.

Column temperature: A constant temperature of about 140°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of D-(+)- $\alpha$ -methylbenzylamine is about 5 minutes.

Selection of column: To 5 mL of D-(+)- $\alpha$ -methylbenzylamine add 1 mL of pyridine. Proceed with 0.6  $\mu$ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pyridine and D-(+)- $\alpha$ -methylbenzylamine in this order with the resolution between these peaks being not less than 3.

Time span of measurement: About 3 times as long as the retention time of D-(+)- $\alpha$ -methylbenzylamine beginning after the solvent peak.

**3-Methyl-1-butanol**  $C_5H_{12}O$  [K 8051, Special class]

**3-Methylbutyl acetate**  $CH_3COOCH_2CH_2CH(CH_3)_2$  A clear and colorless liquid. Boiling point: about 140°C.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.868 – 0.879

Preserve in a light-resistant tight container.

**Methyl cellosolve** See 2-methoxyethanol.

**Methyl docosanoate**  $C_{23}H_{46}O_2$  White, tabular crystals or crystalline powder.

*Melting point* <2.60>: 51.0 – 56.0°C

**Methyldopa** See methyldopa hydrate.

**Methyldopa for assay** See methyldopa hydrate for assay.

**Methyldopa hydrate**  $C_{10}H_{13}NO_4 \cdot 1\frac{1}{2}H_2O$  [Same as the namesake monograph]

**Methyldopa hydrate for assay**  $C_{10}H_{13}NO_4 \cdot 1\frac{1}{2}H_2O$  [Same as the monograph Methyldopa Hydrate. It contains not less than 99.0% of methyldopa ( $C_{10}H_{13}NO_4$ ), calculated on the anhydrous basis.]

***N,N'*-Methylenebisacrylamide**  $CH_2(NHCOCHCH_2)_2$   
White crystalline powder.

*Content*: not less than 97.0%.

**Methylene blue** See methylene blue trihydrate.

**Methylene blue-sulfuric acid-monobasic sodium phosphate TS** See methylene blue-sulfuric acid-sodium dihydrogenphosphate TS.

**Methylene blue-sulfuric acid-sodium dihydrogenphosphate 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 dihydrogenphosphate dihydrate, dissolve, and add water to make 1000 mL.

**Methylene blue trihydrate**  $C_{16}H_{18}ClN_3S \cdot 3H_2O$  [K 8897, Special class]

**Methylene blue TS** Dissolve 0.1 g of methylene blue trihydrate in water to make 100 mL. Filter if necessary.

***dl*-Methylephedrine hydrochloride**  $C_{11}H_{17}NO \cdot HCl$   
[Same as the namesake monograph]

***dl*-Methylephedrine hydrochloride for assay** [Same as the monograph *dl*-Methylephedrine Hydrochloride]

**Methylergometrine maleate for assay**  $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$   
[Same as the monograph Methylergometrine Maleate. When dried, it contains not less than 99.0% of methylergometrine maleate ( $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ ).]

**Methyl ethyl ketone** See 2-butanone.

**Methyl iodide** See iodomethane.

**Methyl iodide for assay** See iodomethane for assay.

**Methyl isobutyl ketone** See 4-methyl-2-pentanone.

**3-O-Methylmethyldopa for thin-layer chromatography**  
 $C_{11}H_{15}NO_4$

*Purity* Related substances—Dissolve 5 mg of 3-O-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL. Perform the test with 20  $\mu$ L of this solution as directed in the Purity (5) under Methyldopa: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.7 does not appear.

**2-Methyl-5-nitroimidazole for thin-layer chromatography**  
 $C_4H_5N_3O_2$  White crystalline powder. Slightly soluble in water and in acetone. Melting point: about 253°C (with decomposition).

*Purity* Related substances—Dissolve 40 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in 8 mL of acetone, and use as the sample solution. Pipet 2.5 mL of the sample solution, add acetone to make exactly 100 mL, and use as the standard solution. Perform the test as directed in the Purity (3) under Metronidazole: the spots other than the

principal spot from the sample solution are not more intense than the spot from the standard solution.

**Methyl orange**  $C_{14}H_{14}N_3NaO_3S$  [K 8893, 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 portions of chloroform.

**Methyl orange TS** Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.

**Methyl orange-xylene cyanol FF TS** Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500 mL of dilute ethanol.

**Methyl parahydroxybenzoate**  $HOC_6H_4COOCH_3$   
[Same as the namesake monograph]

**4-Methylpentan-2-ol**  $C_6H_{14}O$  A clear and colorless, volatile liquid.

*Refractive index* <2.45>  $n_D^{20}$ : about 1.411

*Specific gravity* <2.56>  $d_4^{20}$ : about 0.802

*Boiling point* <2.57>: about 132°C

**4-Methyl-2-pentanone**  $CH_3COCH_2CH(CH_3)_2$   
[K 8903, Special class]

**3-Methyl-1-phenyl-5-pyrazolone**  $C_{10}H_{10}N_2$  [K 9548, Special class]

**Methyl prednisolone**  $C_{22}H_{30}O_3$  [Same as the namesake monograph]

**2-Methyl-1-propanol**  $(CH_3)_2CHCH_2OH$  [K 8811, Special class]

***N*-Methylpyrrolidine**  $C_5H_{11}N$  Colorless, clear liquid, having a characteristic odor.

*Identification*—Determine the spectrum of *N*-methylpyrrolidine in a solution of deuterated chloroform for nuclear magnetic resonance spectroscopy (4 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> ( $^1H$ ): it exhibits a big signal, at around  $\delta$  2.3 ppm.

*Content*: not less than 95%. *Assay*—Put 30 mL of water in a beaker, weigh accurately the beaker, add dropwise about 0.15 g of *N*-methylpyrrolidine, weigh accurately the beaker again, and titrate <2.50> with 0.05 mol/L sulfuric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS  
= 8.515 mg of  $C_5H_{11}N$

**Methyl red**  $C_{15}H_{15}N_3O_2$  [K 8896, 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 100 mL of ethanol (95), and filter if necessary.

**Methyl red TS, dilute** Dissolve 25 mg of methyl red in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

**Methyl red TS for acidity or alkalinity test** To 0.1 g 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.

**Methyrosaniline chloride** See crystal violet.

**Methyrosaniline chloride TS** See crystal violet TS.

**Methyl salicylate**  $C_8H_8O_3$  [Same as the namesake monograph]

**Methylsilicone polymer for gas chromatography** Prepared for gas chromatography.

**Methyltestosterone**  $C_{20}H_{30}O_2$  [Same as the namesake monograph]

**1-Methyl-1H-tetrazole-5-thiol**  $C_2H_4N_4S$  White, crystals or crystalline powder.

**Identification** (1) Determine the ultraviolet-visible absorption spectrum of a solution of 1-methyl-1H-tetrazole-5-thiol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 222 nm and 226 nm.

(2) Determine the infrared absorption spectrum of 1-methyl-1H-tetrazole-5-thiol according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3060\text{ cm}^{-1}$ ,  $2920\text{ cm}^{-1}$ ,  $2780\text{ cm}^{-1}$ ,  $1500\text{ cm}^{-1}$ ,  $1430\text{ cm}^{-1}$  and  $1410\text{ cm}^{-1}$ .

**Melting point** <2.60>: 125 – 129°C

**Purity** Related substances—Dissolve 0.10 g of 1-methyl-1H-tetrazole-5-thiol in exactly 100 mL of water. Perform the test with  $1\text{ }\mu\text{L}$  of this solution as directed in the Purity (4) under Cefmetazole Sodium: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.77 does not appear.

**1-Methyl-1H-tetrazole-5-thiol for liquid chromatography**  $C_2H_4N_4S$  White, crystals or crystalline powder. Very soluble in methanol, and freely soluble in water.

**Melting point** <2.60>: 123 – 127°C

**Loss on drying** <2.41>: not more than 1.0% (1 g, in vacuum, phosphorous (V) oxide, 2 hours).

**Content:** not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 1-methyl-1H-tetrazole-5-thiol, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium methoxide VS  
= 11.61 mg of  $C_2H_4N_4S$

**Methylthymol blue**  $C_{37}H_{43}N_2NaO_{13}S$  [K 9552]

**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 ethylenediaminetetraacetate 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**  $C_{14}H_{15}N_3$  [K 8494, Special class]

**Methyl yellow TS** Dissolve 0.1 g of methyl yellow in 200 mL of ethanol (95).

**Metoclopramide for assay**  $C_{14}H_{22}ClN_3O_2$  [Same as the monograph Metoclopramide. When dried, it contains not less than 99.0% of metoclopramide ( $C_{14}H_{22}ClN_3O_2$ ).]

**Metoprolol tartrate for assay**  $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$  [Same as the monograph Metoprolol Tartrate. When dried, it contains not less than 99.5% of metoprolol tartrate ( $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ ).]

**Metronidazole**  $C_6H_9N_3O_3$  [Same as the namesake monograph]

**Metronidazole for assay**  $C_6H_9N_3O_3$  [Same as the monograph Metronidazole. It meets the following additional requirement.]

**Related substances**—Weigh accurately about 25 mg of metronidazole for assay, dissolve in a mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mixture of water and methanol (4:1) to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly  $10\text{ }\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than metronidazole is not more than the peak area of metronidazole with the standard solution. **Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Metronidazole Tablets.

**Time span of measurement:** About 4 times as long as the retention time of metronidazole.

**System suitability**

**Test for required detectability:** Measure exactly 2 mL of the standard solution, add a mixture of water and methanol (4:1) to make exactly 20 mL. Confirm that the peak area of metronidazole obtained with  $10\text{ }\mu\text{L}$  of this solution is equivalent to 7 to 13% of that with the standard solution.

**System performance:** When the procedure is run with  $10\text{ }\mu\text{L}$  of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with  $10\text{ }\mu\text{L}$  of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 2.0%.

**Miconazole nitrate**  $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$  [Same as the namesake monograph]

**Microplates** Polystyrene plates with an inside diameter of 7 (upper edge) to 6.4 (lower edge) mm, and 11.3 mm thickness. Have 96 flat-bottomed truncated cone-shaped wells.

**Milk casein** See casein, milk.

**Milk of lime** Place 10 g of calcium oxide in a mortar, and add gradually 40 mL of water under grinding.

**Minocycline hydrochloride**  $C_{23}H_{27}N_3O_7 \cdot HCl$  [Same as the namesake monograph]

**Mixture of petroleum hexamethyl tetracosane branching hydrocarbons (L) for gas chromatography** Prepared for gas chromatography.

**Molecular weight markers for teceleukin** Dissolve 0.4 mg each of lysozyme, soy trypsin inhibitor, carbonic anhydrase, egg white albumin, bovine serum albumin, and phosphorylase b in 200  $\mu$ L of diluted glycerin (1 in 2).

**Molybdenum (III) oxide**  $MoO_3$  A white to yellowish green powder.

*Identification*—Dissolve 0.5 g in 5 mL of ammonia solution (28), acidify 1 mL of this solution with a suitable amount of nitric acid, add 5 mL of sodium phosphate TS, and warm: yellow precipitates appear.

**Molybdenum (III) oxide-citric acid TS** To 54 g of molybdenum (III) oxide and 11 g of sodium hydroxide add 200 mL of water, and dissolve by heating while stirring. Separately, dissolve 60 g of citric acid monohydrate in 250 mL of water, and add 140 mL of hydrochloric acid. Mix these solutions, filter if necessary, add water to make 1000 mL, and add a solution of potassium bromate (1 in 100) until a yellow-green color appears.

*Storage*—Preserve in tightly stoppered containers, protected from light.

**Molybdenum trioxide** See molybdenum (III) oxide.

**Molybdenum trioxide-citric acid TS** See molybdenum (III) oxide-citric acid TS.

**Monobasic ammonium phosphate** See ammonium dihydrogenphosphate.

**0.02 mol/L Monobasic ammonium phosphate TS** See 0.02 mol/L ammonium dihydrogenphosphate TS.

**Monobasic potassium phosphate** See potassium dihydrogenphosphate.

**Monobasic potassium phosphate for pH determination** See potassium dihydrogenphosphate for pH determination.

**0.05 mol/L Monobasic potassium phosphate, pH 3.0** See 0.05 mol/L potassium dihydrogenphosphate, pH 3.0.

**0.05 mol/L Monobasic potassium phosphate TS, pH 4.7** See 0.05 mol/L potassium dihydrogenphosphate TS, pH 4.7.

**0.02 mol/L Monobasic potassium phosphate TS** See 0.02 mol/L potassium dihydrogenphosphate TS.

**0.05 mol/L Monobasic potassium phosphate TS** See 0.05 mol/L potassium dihydrogenphosphate TS.

**0.2 mol/L Monobasic potassium phosphate TS** See 0.2 mol/L potassium dihydrogenphosphate TS.

**0.2 mol/L Monobasic potassium phosphate TS for buffer solution** See 0.2 mol/L potassium dihydrogenphosphate TS for buffer solution.

**Monobasic sodium phosphate** See sodium dihydrogen-

phosphate dihydrate.

**0.05 mol/L Monobasic sodium phosphate TS, pH 2.6** See 0.05 mol/L sodium dihydrogenphosphate TS, pH 2.6.

**0.05 mol/L Monobasic sodium phosphate TS, pH 3.0** See 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0.

**0.1 mol/L Monobasic sodium phosphate TS, pH 3.0** See 0.1 mol/L sodium dihydrogenphosphate TS, pH 3.0.

**0.05 mol/L Monobasic sodium phosphate TS** See 0.05 mol/L sodium dihydrogenphosphate TS.

**0.1 mol/L Monobasic sodium phosphate TS** See 0.1 mol/L sodium dihydrogenphosphate TS.

**2 mol/L Monobasic sodium phosphate TS** See 2 mol/L sodium dihydrogenphosphate TS.

**Monoethanolamine** See 2-Aminoethanol.

**Morphine hydrochloride** See morphine hydrochloride hydrate.

**Morphine hydrochloride for assay** See morphine hydrochloride hydrate for assay.

**Morphine hydrochloride hydrate**  $C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$  [Same as the namesake monograph]

**Morphine hydrochloride hydrate for assay**  $C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$  [Same as the monograph Morphine Hydrochloride Hydrate. It contains not less than 99.0% of morphine hydrochloride ( $C_{17}H_{19}NO_3 \cdot HCl$ ), calculated on the anhydrous basis.]

**3-(N-Morpholino)propanesulfonic acid**  $C_7H_{15}NO_4S$  White crystalline powder, freely soluble in water, and practically insoluble in ethanol (99.5).

*Melting point* <2.60>: 275 – 280°C

**0.02 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0** Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0** Dissolve 20.92 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

**0.02 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 8.0** Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 700 mL of water, adjust the pH to 8.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

**Mosapride citrate for assay** See mosapride citrate hydrate for assay.

**Mosapride citrate hydrate for assay**  $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7 \cdot 2H_2O$  [Same as the monograph Mosapride Citrate Hydrate. It contains not less than 99.0% of mosapride citrate ( $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$ ) calculated on the anhydrous basis.]

**MTT TS** Dissolve 8 g of sodium chloride, 0.2 g of calcium chloride, 1.15 g of anhydrous sodium dihydrogen phosphate ( $NaH_2PO_4$ ) and 0.2 g of potassium dihydrogen phosphate ( $KH_2PO_4$ ) in water to make 1000 mL, and sterilize in

an autoclave for 15 minutes at 121°C to make the PBS(-) solution. Dissolve 0.3 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide in this PBS(-) solution to make 100 mL. Sterilize by membrane filtration (pore size, 0.45 μm), and store in a cool place shielded from light.

**Murexide** C<sub>8</sub>H<sub>8</sub>N<sub>6</sub>O<sub>6</sub> Red-purple powder. Practically insoluble in water, in ethanol (95) and in diethyl ether.

**Purity** Clarity of solution—Dissolve 10 mg of murexide in 100 mL of water: the solution is clear.

**Residue on ignition** <2.44>: not more than 0.1% (1 g).

**Sensitivity**—Dissolve 10 mg 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 sample 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 sample 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.

**Myoglobin** A hemoprotein obtained from horse heart muscle. White crystalline powder. It contains not less than 95% of myoglobin in the total protein.

**Myristicin for thin-layer chromatography** C<sub>11</sub>H<sub>12</sub>O<sub>3</sub> Colorless, clear liquid, having a characteristic odor. Miscible with ethanol (95), and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum of myristicin for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3080 cm<sup>-1</sup>, 2890 cm<sup>-1</sup>, 1633 cm<sup>-1</sup>, 1508 cm<sup>-1</sup>, 1357 cm<sup>-1</sup>, 1318 cm<sup>-1</sup>, 1239 cm<sup>-1</sup>, 1194 cm<sup>-1</sup>, 1044 cm<sup>-1</sup>, 994 cm<sup>-1</sup>, 918 cm<sup>-1</sup>, 828 cm<sup>-1</sup> and 806 cm<sup>-1</sup>.

**Purity** Related substances—Dissolve 20 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 0.5 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification under Nutmeg: the spots other than the principle spot at the R<sub>f</sub> value of about 0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

**Nalidixic acid** C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> [Same as the namesake monograph]

**Naphazoline nitrate** C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>.HNO<sub>3</sub> [Same as the namesake monograph]

**Naphazoline nitrate for assay** C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>.HNO<sub>3</sub> [Same as the monograph Naphazoline Nitrate. When dried, it contains not less than 99.0% of naphazoline nitrate (C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>.NHO<sub>3</sub>).]

**Naphthalene** C<sub>10</sub>H<sub>8</sub> Colorless flake-like or lustrous stick-like crystals, having a characteristic odor.

**Melting point** <2.60>: 78 – 82°C

**1,3-Naphthalenediol** C<sub>10</sub>H<sub>8</sub>O<sub>2</sub> Red-brown crystals or

gray-brown powder. Freely soluble in water, in methanol and in ethanol (99.5). Melting point: about 124°C.

**1,3-Naphthalenediol TS** Dissolve 50 mg of 1,3-naphthalenediol in 25 mL of ethanol (99.5), and add 2.5 mL of phosphoric acid.

**2-Naphthalenesulfonic acid** See 2-naphthalenesulfonic acid hydrate.

**2-Naphthalenesulfonic acid monohydrate** C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>.H<sub>2</sub>O White to pale yellowish white powder. Very soluble in water, in methanol and in ethanol (95), and sparingly soluble in diethyl ether and in chloroform.

**Water** <2.48>: 7.0 – 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 hydrate, dissolve in 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 20.82 mg of C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>S

**1-Naphthol** C<sub>10</sub>H<sub>7</sub>OH [K 8698, Special class] Preserve in light-resistant containers.

**2-Naphthol** C<sub>10</sub>H<sub>7</sub>OH [K 8699, Special class] Preserve in light-resistant containers.

**α-Naphthol** See 1-naphthol.

**β-Naphthol** See 2-naphthol.

**p-Naphtholbenzein** C<sub>27</sub>H<sub>20</sub>O<sub>3</sub> [K 8693, Special class]

**α-Naphtholbenzein** See p-naphtholbenzein.

**p-Naphtholbenzein TS** Dissolve 0.2 g of p-naphtholbenzein in acetic acid (100) to make 100 mL.

**Purity** Clarity and color of solution—Dissolve 0.1 g of p-naphtholbenzein in 100 mL of ethanol (95): the solution is red in color and clear.

**Sensitivity**—Add 100 mL of freshly boiled and cooled water to 0.2 mL of a solution of p-naphtholbenzein in ethanol (95) (1 in 1000), and add 0.1 mL 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-Naphtholbenzein TS** See p-naphtholbenzein TS.

**1-Naphthol-sulfuric acid TS** Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid, and mix well. Prepare before use.

**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 100 mL. Prepare before use.

**α-Naphthol TS** See 1-naphthol TS.

**β-Naphthol TS** See 2-naphthol TS.

**1-Naphthylamine** C<sub>10</sub>H<sub>7</sub>NH<sub>2</sub> [K 8692, Special class] Preserve in light-resistant containers.

**$\alpha$ -Naphthylamine** See 1-naphthylamine.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate** See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS** See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS**  
See *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS.

***N*-1-Naphthylethylenediamine dihydrochloride**  
 $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$  [K 8197, Special class]

**Naphthylethylenediamine TS** Dissolve 0.1 g of *N*-1-naphthylethylenediamine dihydrochloride in water to make 100 mL. Prepare before use.

**Naringin for thin-layer chromatography** See naringin dihydrate for thin-layer chromatography.

**Naringin dihydrate for thin-layer chromatography**  
 $C_{27}H_{32}O_{14} \cdot 2H_2O$  White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and slightly soluble in water. Melting point: about 170°C (with decomposition).

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : -87 - -93° (0.1 g, ethanol (95), 10 mL, 100 mm).

**Purity** Related substances—Proceed with 10  $\mu$ L of a solution, prepared by dissolving 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), as directed in the Identification under Bitter Orange Peel: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Neocarzinostatin**  $C_{511}H_{768}N_{132}O_{179}S_4$  A white or pale yellowish white powder.

**Identification**—Determine the absorption spectrum of a solution of the substance to be examined (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 270 nm and 275 nm, and shoulders between 288 nm and 292 nm and between 330 nm and 360 nm.

**Purity**—Dissolve 10 mg of the substance to be examined in the mobile phase to make 50 mL, and use this as the sample solution. Perform the test with 0.25 mL of the sample solution as directed under Liquid Chromatography <2.01>, determine each peak area by the automatic integration method, and calculate the amount of neocarzinostatin by the area percentage method: not less than 90.0%.

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 254 nm).

**Column:** Pre-column is a stainless steel column 7.5 mm in inside diameter and 75 mm in length, packed with silica gel for liquid chromatography (10  $\mu$ m in particle size). Separation column is a stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with silica gel for liquid chromatography (10  $\mu$ m in particle size), which is coupled to the pre-column.

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** Dissolve 3.78 g of potassium dihydrogen phosphate and 5.52 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

**Flow rate:** Adjust the flow rate so that the retention time

of neocarzinostatin is about 21 minutes.

**Time span of measurement:** About 2 times as long as the retention time of neocarzinostatin.

**System suitability**

**System performance:** When the procedure is run with 0.25 mL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of neocarzinostatin are not less than 2000 and not more than 2.5, respectively.

**System repeatability:** When the test is repeated 6 times with 0.25 mL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of neocarzinostatin is not more than 2.0%.

**Water** <2.48> Not more than 10.0% (10 mg, coulometric titration).

**Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)** An amido binding condensate of neocarzinostatin and styrene-maleic acid alternating copolymer partial butyl ester in a rate of 2:3. Average molecular mass: about 28,400. A pale yellow powder.

**Identification**—Dissolve 4 mg of the substance to be examined in 0.05 mol/L phosphate buffer solution, pH 7.0 to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 266 nm and 270 nm, and shoulders between 257 nm and 262 nm, between 286 nm and 291 nm and between 318 nm and 348 nm.

**Absorbance** <2.24>  $E_{1\%}^{1\text{cm}}$  (268 nm): 13.0 - 17.5 [4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL]

**Purity**—Proceed as directed in the Purity (3) under Zinostatin Stimalamer, with the exception of without using of (iii) Standard solution, and changing (iv) Sample solution, (v) Procedure and (vii) Determination as follows:

(iv) **Sample solution** Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 10 mL.

(v) **Procedure** Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100  $\mu$ L of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vii) **Determination** Determine the peak area,  $A_T$ , of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) and the total area,  $A$ , of the peaks other than neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formula: not less than 90.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of neocarzinostatin-styrene-maleic acid} \\ \text{alternating copolymer partial butyl ester condensate (2:3)} \\ = \{A_T / (A_T + A)\} \times 100 \end{aligned}$$

**Water** <2.48> Not more than 12.0% (10 mg, coulometric titration).

**Neutral alumina containing 4% of water** Take 50 g of neutral alumina for column chromatography, previously dried at 105°C for 2 hours, in a tight container, add 2.0 mL of water, shake well to make homogeneous, and allow to stand for more than 2 hours.

**Neutral detergent** Synthetic detergent containing anionic or non-ionic surfactant, and pH of its 0.25% solution is between 6.0 and 8.0. Dilute to a suitable concentration before use.

**Neutralized ethanol** See ethanol, neutralized.

**Neutral red**  $C_{15}H_{17}N_4Cl$  Slightly metallic, dark green powder or masses.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3310\text{ cm}^{-1}$ ,  $3160\text{ cm}^{-1}$ ,  $1621\text{ cm}^{-1}$ ,  $1503\text{ cm}^{-1}$ ,  $1323\text{ cm}^{-1}$ ,  $1199\text{ cm}^{-1}$  and  $732\text{ cm}^{-1}$ .

**Neutral red TS** Dissolve 0.1 g of neutral red in acetic acid (100) to make 100 mL.

**Nicardipine hydrochloride for assay**  $C_{26}H_{29}N_3O_6.HCl$  [Same as the monograph Nicardipine Hydrochloride. When dried, it contains not less than 99.0% of nicardipine hydrochloride ( $C_{26}H_{29}N_3O_6.HCl$ ).]

**Nicergoline for assay**  $C_{24}H_{26}BrN_3O_3$  [Same as the monograph Nicergoline, or Nicergoline purified according to the method below. When dried, it contains not less than 99.0% of nicergoline ( $C_{24}H_{26}BrN_3O_3$ ), and when perform the test of the Purity (2) under Nicergoline, the total area of the peaks other than nicergoline from the sample solution is not more than 2.5 times the peak area of nicergoline from the standard solution.

Method of purification: Dissolve 1 g of Nicergoline in 20 mL of acetonitrile, allow to stand in a dark place for about 36 hours, filter, and dry the crystals so obtained at 60°C for 2 hours in vacuum.]

**Nicomol for assay**  $C_{34}H_{32}N_4O_9$  [Same as the monograph Nicomol. When dried, it contains not less than 99.0% of  $C_{34}H_{32}N_4O_9$ .]

**Nicotinamide**  $C_6H_6N_2O$  [Same as the namesake monograph]

**$\beta$ -Nicotinamide-adenine dinucleotide ( $\beta$ -NAD)**  
 $C_{21}H_{27}N_7O_{14}P_2$  [K 9802]

**Content:** not less than 94.5%. **Assay**—Weigh accurately about 25 mg of  $\beta$ -nicotinamide-adenine dinucleotide, oxidized form, and dissolve in water to make exactly 25 mL. Pipet 0.2 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10 mL, and use this solution as the sample solution. Determine the absorbances,  $A_T$  and  $A_B$ , of the sample solution and 0.1 mol/L phosphate buffer solution, pH 7.0, at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\begin{aligned} & \text{Amount (mg) of } C_{21}H_{27}N_7O_{14}P_2 \\ &= \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_T - A_B) \times 25 \end{aligned}$$

**$\beta$ -Nicotinamide adenine dinucleotide TS** Dissolve 40 mg of  $\beta$ -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]

**Nile blue**  $C_{20}H_{20}ClN_3O$  Blue-green powder.

**Ninhydrin**  $C_9H_6O_4$  [K 8870, Special class]

**Ninhydrin TS** Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

**Ninhydrin-acetic acid TS** Dissolve 1.0 g of ninhydrin in 50 mL of ethanol (95), and add 10 mL of acetic acid (100).

**Ninhydrin-L-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-butanol TS** Dissolve 0.3 g of ninhydrin in 100 mL of 1-butanol, and add 3 mL of acetic acid (100).

**Ninhydrin-citric acid-acetic acid TS** Dissolve 70 g of citric acid monohydrate in 500 mL of water, add 58 mL of acetic acid (100), 70 mL of a solution of sodium hydroxide (21 in 50) and water to make 1000 mL. In 100 mL of this solution dissolve 0.2 g of ninhydrin.

**Ninhydrin-stannous chlorid TS** See ninhydrin-tin (II) chloride TS.

**Ninhydrin-sulfuric acid TS** Dissolve 0.1 g of ninhydrin in 100 mL of sulfuric acid. Prepare before use.

**Ninhydrin-tin (II) chloride TS** Dissolve 21.0 g of citric acid in water to make 200 mL, adjust the pH to  $5.6 \pm 0.2$  by adding sodium hydroxide TS, add water to make 500 mL, and dissolve 1.3 g of tin (II) chloride. To 50 mL of the solution, add 50 mL of a 2-methoxyethanol 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.

**Nitrendipine for assay** [Same as the monograph Nitrendipine. It, when dried, contains not less than 99.0% of nitrendipine ( $C_{18}H_{20}N_2O_6$ ), and meets the following requirement. When perform the test as directed in the Purity (2) under Nitrendipine, the area of the peak of dimethyl ester, having the relative retention time of about 0.8 with respect to nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution, the area of the peak other than nitrendipine and the dimethyl ester is not larger than 1/5 times the peak area of nitrendipine from the standard solution, and the total area of the peak other than nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution.]

**Nitric acid**  $HNO_3$  [K 8541, Special class, Concentration: 69 – 70%, Density: about 1.42 g/mL]

**Nitric acid, dilute** Dilute 10.5 mL of nitric acid with water to make 100 mL (10%).

**Nitric acid, fuming** [K 8739, Special class, Concentration: not less than 97%, Density: 1.52 g/mL]

**Nitric acid TS, 2 mol/L** Dilute 12.9 mL of nitric acid

with water to make 100 mL.

**2,2',2''-Nitrilotriethanol**  $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}$  [K 8663, Special class]

**2,2',2''-Nitrilotriethanol buffer solution, pH 7.8**

Dissolve 149.2 g of 2,2',2''-nitrilotriethanol in about 4500 mL of water, adjust to pH 7.8 with diluted 6 mol/L hydrochloric acid TS (2 in 3), and add water to make 5000 mL.

**3-Nitroaniline**  $\text{C}_6\text{H}_5\text{N}_2\text{O}_2$  Yellow crystals or crystalline powder.

*Melting point* <2.60>: 112 – 116°C

**4-Nitroaniline**  $\text{C}_6\text{H}_4\text{NO}_2\text{NH}_2$  Yellow to yellowish-red crystals or crystalline powder.

*Melting point* <2.60>: 147 – 150°C.

Preserve in a light-resistant tight container.

**p-Nitroaniline** See 4-nitroaniline.

**p-Nitroaniline-sodium nitrite TS** See 4-nitroaniline-sodium nitrite TS.

**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** See 2-nitrobenzaldehyde.

**2-Nitrobenzaldehyde**  $\text{O}_2\text{NC}_6\text{H}_4\text{CHO}$  Pale yellow crystals or crystalline powder.

*Melting point* <2.60>: 42 – 44°C

**Nitrobenzene**  $\text{C}_6\text{H}_5\text{NO}_2$  [K 8723, Special class]

**p-Nitrobenzenediazonium chloride TS** See 4-nitrobenzenediazonium chloride TS.

**4-Nitrobenzenediazonium 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.

**p-Nitrobenzenediazonium fluoroborate** See 4-nitrobenzenediazonium fluoroborate.

**4-Nitrobenzenediazonium fluoroborate**

$\text{O}_2\text{NC}_6\text{H}_4\text{N}_2\text{BF}_4$  Pale yellowish white, almost odorless powder. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly soluble in ethanol (95) and in chloroform. *Melting point*: about 148°C (with decomposition).

*Identification*—Add 1 mL 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.

*Loss on drying* <2.41>: not more than 1.0% (1 g, silica gel, 2 hours).

**p-Nitrobenzoyl chloride** See 4-nitrobenzoyl chloride.

**4-Nitrobenzoyl chloride**  $\text{O}_2\text{NC}_6\text{H}_4\text{COCl}$  Light yellow crystals.

*Melting point* <2.60>: 70 – 74°C

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 0.5 g of 4-nitrobenzoyl chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash with water, dry at 105°C to constant mass, and weigh. The mass of 4-nitrobenzoyl chloride, multiplied by 1.107, represents the mass of 4-nitrobenzoyl chloride ( $\text{C}_7\text{H}_4\text{ClNO}_3$ ).

**p-Nitrobenzyl chloride** See 4-nitrobenzyl chloride.

**4-Nitrobenzyl chloride**  $\text{O}_2\text{NC}_6\text{H}_4\text{CH}_2\text{Cl}$  Light yellow crystals or crystalline powder. Soluble in ethanol (95).

*Melting point* <2.60>: 71 – 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 on a water bath under a reflex condenser for 1 hour. After cooling, filter the precipitate with a glass filter, wash with water, dry at 105°C to constant mass, and weigh. The mass of the precipitate represents the amount of silver chloride (AgCl: 143.32).

Amount (mg) of 4-nitrobenzyl chloride

= amount (mg) of silver chloride (AgCl: 143.32)  $\times$  1.197

**4-(4-Nitrobenzyl)pyridine**  $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2$  Pale yellow, crystalline powder. Freely soluble in acetone, and soluble in ethanol (95).

*Melting point* <2.60>: 69 – 71°C

**Nitroethane**  $\text{C}_2\text{H}_5\text{NO}_2$

*Density* <2.56>: 1.048 – 1.053 g/cm<sup>3</sup> (20°C)

*Water* <2.48>: not more than 0.1%.

**Nitrogen** [Same as the namesake monograph]

**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**  $\text{CH}_3\text{NO}_2$  [K 9523, Special class]

**3-Nitrophenol**  $\text{C}_6\text{H}_5\text{NO}_3$  A light yellow crystalline powder.

*Melting point* <2.60>: 96 – 99°C

**4-Nitrophenol**  $\text{C}_6\text{H}_5\text{NO}_3$  [K 8721, Special class]

**o-Nitrophenyl- $\beta$ -D-galactopyranoside** See 2-nitrophenyl- $\beta$ -D-galactopyranoside.

**2-Nitrophenyl- $\beta$ -D-galactopyranoside**  $\text{C}_{12}\text{H}_{15}\text{NO}_8$

White crystalline powder. Odorless. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

*Melting point* <2.60>: 193 – 194°C

*Purity* Clarity and color of solution—A solution of 2-nitrophenyl- $\beta$ -D-galactopyranoside (1 in 100) is clear and colorless.

*Loss on drying* <2.41>: not 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 2-nitrophenyl- $\beta$ -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 Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} &\text{Amount (mg) of 2-nitrophenyl-}\beta\text{-D-galactopyranoside} \\ &= \frac{A}{133} \times 25,000 \end{aligned}$$

**1-Nitroso-2-naphthol**  $C_{10}H_7NO_2$  A yellow-brown to red-brown crystalline powder.

*Melting point* <2.60>: 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.

**$\alpha$ -Nitroso- $\beta$ -naphthol** See 1-nitroso-2-naphthol.

**$\alpha$ -Nitroso- $\beta$ -naphthol TS** See 1-nitroso-2-naphthol TS.

**Nitrous oxide**  $N_2O$  Colorless and odorless gas. Use nitrous oxide from a metal cylinder.

**NK-7 cells** Cells derived from mouse NK cells.

**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.

**Nodakenin for thin-layer chromatography**  $C_{20}H_{24}O_9$  White powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 220°C (with decomposition).

*Identification*—Determine the absorption spectrum of a solution of nodakenin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 333 nm and 337 nm.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +50 – +68° (5 mg, methanol, 10 mL, 100 mm).

*Purity* Related substances—Dissolve 1 mg of nodakenin for thin-layer chromatography in 3 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5  $\mu$ L each of these solutions as directed in the Identification (2) under Peucedanum Root: the spot other than the principal spot of around  $R_f$  value of 0.3 from the sample solution is not more intense than the spot from the standard solution.

**Nonylphenoxypoly(ethyleneoxy)ethanol for gas chromatography** Prepared for gas chromatography.

**Normal agar media for teceleukin** Dissolve 5.0 g of meat extract, 10.0 g of peptone, 5.0 g of sodium chloride, and 15.0 to 20.0 g of agar in water to make 1000 mL, and sterilize. Adjust the pH to 6.9 to 7.1.

**$n$ -Octadecane**  $C_{18}H_{38}$  Colorless or white solid at ordinary temperature.

*Purity* Clarity of solution—A solution of  $n$ -octadecane in chloroform (1 in 25) is clear.

**Octadecylsilanized silica gel for pretreatment** Prepared

for pretreatment.

**$n$ -Octane**  $C_8H_{18}$

*Specific gravity* <2.56>  $d_4^{20}$ : 0.700 – 0.705

*Purity*—Perform the test with 2  $\mu$ L of  $n$ -octane as directed under Gas Chromatography <2.02> according to the conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of  $n$ -octane by the area percentage method: not less than 99.0%.

**Octane, iso** A colorless liquid. Practically insoluble in water. Miscible with diethyl ether and with chloroform.

*Purity*—Determine the absorbances of isooctane at 230 nm, 250 nm and 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution: these values are not more than 0.050, 0.010 and 0.005, respectively.

**1-Octanol**  $CH_3(CH_2)_6CH_2OH$  [K 8213, Special class]

**Octyl alcohol** See 1-octanol.

**$n$ -Octylbenzene**  $C_{14}H_{22}$  Clear and colorless liquid, having a characteristic odor.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.854 – 0.863

*Distillation test* <2.57>: 263 – 265°C, not less than 95 vol%.

**Ofloxacin**  $C_{18}H_{20}FN_3O_4$  [Same as the namesake monograph]

**Ofloxacin demethyl substance** ( $\pm$ )-9-Fluoro-2,3-dihydro-3-methyl-7-oxo-7H-10-(1-piperazinyl)-pirido[1, 2, 3-*de*] [1, 4]benzoxazine-6-carboxylic acid  $C_{17}H_{18}FN_3O_4$  White to light green-yellowish white, crystals or crystalline powder.

*Identification*—Determine the infrared absorption spectrum of ofloxacin demethyl substance as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3050  $cm^{-1}$ , 2840  $cm^{-1}$ , 1619  $cm^{-1}$ , 1581  $cm^{-1}$ , 1466  $cm^{-1}$ , 1267  $cm^{-1}$ , 1090  $cm^{-1}$ , 1051  $cm^{-1}$  and 816  $cm^{-1}$ .

**Oleic acid**  $C_{18}H_{34}O_2$  Occurs as a colorless or pale yellow transparent liquid and has a slightly distinct odor. It is miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : about 0.9

*Content*: not less than 99.0%. Assay—To 40  $\mu$ L of oleic acid to be examined add 1 mL of a solution of boron trifluoride in methanol (3 in 20), mix, and heat on a water bath for 3 minutes. After cooling, add 10 mL of petroleum ether and 10 mL of water, shake, collect the ether layer after allowing to stand, and use as the sample solution. Perform the test with 0.2  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of methyl oleate by the area percentage method.

*Operating conditions*

Detector: A hydrogen flame-ionization detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (149 – 177  $\mu$ m) coated with methyl polyacrylate in a rate of 5 – 10%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of methyl oleate is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of methyl oleate, beginning after the solvent peak.

**Olive oil** [Same as the namesake monograph]

**Ophiopogon tuber** [Same as the namesake monograph]

**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), and in diethyl ether.

*Melting point* <2.60>: 107 – 111°C

**Orcine-ferric chloride TS** See orcine-iron (III) chloride TS.

**Orcine-iron (III) chloride TS** Dissolve 10 mg of orcine in 1 mL 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 the loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the filtrate, and sterilize by autoclaving. When powdered agar is used, 15 to 20 g of it is dissolved.

**Ordinary broth** Dissolve 5 g of beef extract and 10 g of peptone in 1000 mL 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 the loss, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121°C.

**Osthole for thin-layer chromatography**  $C_{15}H_{16}O_3$  A white crystalline powder, having no odor. Freely soluble in methanol and in ethyl acetate, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 83 – 84°C.

*Purity* Related substances—Dissolve 1.0 mg of osthole for thin-layer chromatography in 1 mL of methanol. Perform the test with 10  $\mu$ L of this solution as directed in the Identification under *Cnidium Monnieri* Fruit: on spot appears other than the principal spot at around *Rf* 0.3.

**Oxalate pH standard solution** See pH Determination <2.54>.

**Oxalic acid** See oxalic acid dihydrate.

**Oxalic acid dihydrate**  $H_2C_2O_4 \cdot 2H_2O$  [K 8519, Special class]

**Oxalic acid TS** Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

**Oxycodone hydrochloride for assay** See oxycodone hydrochloride hydrate for assay.

**Oxycodone hydrochloride hydrate for assay**  $C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$  [Same as the monograph Oxycodone Hydrochloride Hydrate. It contains not less than 99.0% of oxycodone hydrochloride ( $C_{18}H_{21}NO_4 \cdot HCl$ ), calculated on the anhydrous basis.]

**Oxygen**  $O_2$  [K 1101]

**2-Oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid** See 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid.

**8-Oxyquinoline** See 8-quinolinol.

**Oxytocin**  $C_{43}H_{66}N_{12}O_{12}S_2$  [Same as the namesake monograph]

**Paeoniflorin for thin-layer chromatography**

$C_{23}H_{28}O_{11} \cdot xH_2O$  Colorless, odorless powder. Freely soluble in water and in methanol, and not dissolves in diethyl ether. Melting point: 123 – 125°C (with decomposition).

*Purity* Related substances—Dissolve 1.0 mg of paeoniflorin for thin layer chromatography in exactly 1 mL of methanol. Perform the test with 20  $\mu$ L of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

**Paeonol for assay**  $C_9H_{10}O_3$  Use paeonol for thin-layer chromatography meeting the following additional specifications.

*Absorbance* <2.24>  $E_{1\%}^{1\text{cm}}$  (274 nm): 853 – 934 [5 mg after drying in a desiccator (calcium chloride for drying) for 1 hour or more, methanol, 1000 mL].

*Purity* Related substances—Dissolve 5.0 mg of paeonol for assay in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than paeonol from the sample solution is not larger than the peak area of paeonol from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Moutan Bark except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of paeonol obtained from 10  $\mu$ L of the standard solution (2) can be measured, and the peak height of paeonol from 10  $\mu$ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of paeonol beginning after the solvent peak.

**Paeonol for component determination** See paeonol for assay.

**Paeonol for thin-layer chromatography**  $C_9H_{10}O_3$  White, crystals or crystalline powder, having a specific odor. Freely soluble in methanol and in diethyl ether, and slightly soluble in water. Melting point: about 50°C

*Purity* Related substances—Dissolve 1.0 mg of paeonol for thin-layer chromatography in exactly 1 mL of methanol, and perform with 10  $\mu$ L of this solution as directed in the Identification under Moutan Bark: any spot other than the principal spot at the *Rf* value of near 0.5 does not appear.

**Palladium chloride** See palladium (II) chloride.

**Palladium chloride TS** See palladium (II) chloride TS.

**Palladium (II) chloride**  $PdCl_2$  [K 8154, 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, by heating if necessary, cool, and add 0.25 mol/L sulfuric acid TS to make 1000 mL.

**Palmatin chloride**  $C_{21}H_{22}ClNO_4 \cdot xH_2O$  A yellow-brown crystalline powder.

**Purity**—Dissolve 1 mg of palmatin chloride in 10 mL of methanol, and use this solution as the sample solution. Proceed with 20  $\mu$ L of the sample solution as directed in the Assay under Phellodendron Bark: when measure the peak areas 2 times as long as the retention time of palmatin, the total area of the peaks other than palmatin is not larger than 1/10 of the total area except the area of solvent peak.

**Palmitic acid for gas chromatography**  $C_{16}H_{32}O_2$  [K 8756, Special class]

**Pancreatic digest of casein** See Peptone, casein.

**Pancreatic digest of gelatin** See Peptone, gelatin.

**Papaic digest of soya bean** See Peptone, soybean.

**Papaverine hydrochloride**  $C_{20}H_{21}NO_4 \cdot HCl$  [Same as the namesake monograph]

**Papaverine hydrochloride for assay**  $C_{20}H_{21}NO_4 \cdot HCl$  [Same as the monograph Papaverine Hydrochloride. When dried, it contains not less than 99.0% of papaverine hydrochloride ( $C_{20}H_{21}NO_4 \cdot HCl$ ).]

**Paraffin** [Same as the namesake monograph]

**Paraffin, light liquid** [Same as the namesake monograph]

**Parahydroxybenzoic acid**  $C_7H_6O_3$  White crystals.

**Melting point** <2.60>: 212 – 216°C

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.7 g of parahydroxybenzoic acid, dissolve in 50 mL of acetone, add 100 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS  
= 69.06 mg of  $C_7H_6O_3$

**PBS containing bovine serum** To 100 mL of bovine serum add 900 mL of phosphate-buffered sodium chloride TS containing thimerosal (0.1 g) to make 1000 mL.

**Storage**—Store in a cool place shielded from light.

**PBS containing bovine serum albumin** Add phosphate-buffered sodium chloride TS to 10 g of bovine serum albumin and 0.1 g of thimerosal to make 1000 mL.

**Storage**—Store in a cool, dark place.

**Peanut oil** [Same as the namesake monograph]

**Pentane**  $CH_3(CH_2)_3CH_3$  Clear and colorless liquid.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 0.620 – 0.650

**Distilling range** <2.57>: 35.5 – 37°C, not less than 98 vol%.

**Peptic digest of animal tissue** See Peptone, animal tissue.

**Peptone** Prepared for microbial test.

**Peptone, animal tissue** Prepared for microbial test.

**Peptone, casein** Grayish yellow powder, having a characteristic but not putrescent odor. It dissolves in water, but not in ethanol (95) and in diethyl ether.

**Loss on drying** <2.41>: not more than 7% (0.5 g, 105°C, constant mass).

**Residue on ignition** <2.44>: not more than 15% (0.5 g).

**Degree of digestion**—Dissolve 1 g of casein peptone in 10 mL of water, and perform the following test using this solution as the sample solution:

(1) Overlay 1 mL of the sample 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 1 mL of the sample solution with 4 mL of a saturated solution of zinc sulfate heptahydrate: 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** <1.08>: not less than 10% (105°C, constant mass, after drying).

**Peptone, gelatin** Prepared for microbial test.

**Peptone, soybean** Prepared for microbial test.

**Perchloric acid**  $HClO_4$  [K 8223, Special class, Density: about 1.67 g/mL. Concentration: 70.0 – 72.0%]

**Perchloric acid-dehydrated ethanol TS** See perchloric acid-ethanol TS.

**Perchloric acid-ethanol TS** Add cautiously 25.5 mL of perchloric acid to 50 mL of ethanol (99.5), cool, and add ethanol (99.5) to make 100 mL (3 mol/L).

**Performic acid** Mix 9 volumes of formic acid and 1 volume of hydrogen peroxide (30), and leave at room temperature for 2 hours.

**Storage**—Store in a cool place.

**Perillaldehyde for assay** Perillaldehyde for thin-layer chromatography meeting the following specifications.

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (230 nm): 850 – 950 (10 mg, methanol, 2000 mL).

**Purity** Related substances—Dissolve 10 mg of perillaldehyde in 250 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of perillaldehyde from the sample solution is not larger than the peak of perillaldehyde from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Perilla Herb.

Time span of measurement: About 3 times as long as the retention time of perillaldehyde beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard

solution, and add methanol to make exactly 20 mL. Confirm that the peak area of perillaldehyde obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that of perillaldehyde from 10  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Perilla Herb.

**Perillaldehyde for component determination** See perillaldehyde for assay.

**Perillaldehyde for thin-layer chromatography**  $C_{10}H_{14}O$  Colorless to light brown transparent liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and very slightly soluble in water.

**Identification**—Determine the infrared absorption spectrum of perillaldehyde for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3080  $cm^{-1}$ , 2930  $cm^{-1}$ , 1685  $cm^{-1}$ , 1644  $cm^{-1}$ , 1435  $cm^{-1}$  and 890  $cm^{-1}$ .

**Purity** Related substances—Dissolve 1.0 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification under Perilla Herb: no spot other than the principal spot at around *R<sub>f</sub>* value of 0.5 appears.

**Peroxidase** Obtained from horse-radish. A red-brown powder. It is freely soluble in water. It contains about 250 units per mg. One unit indicates an amount of the enzyme which produces 1 mg of purpurogallin in 20 seconds at 20°C and pH 6.0, from pyrogallol and hydrogen peroxide (30) used as the substrate.

**Peroxidase-labeled antibody stock solution** 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS containing antibody fragment (Fab') bound to peroxidase.

**Peroxidase-labeled bradykinin** A solution of horseradish origin peroxidase-binding bradykinin in gelatin-phosphate buffer solution, pH 7.0. A colorless to light brown clear solution.

**Peroxidase-labeled bradykinin TS** To 0.08 mL of peroxidase-labeled bradykinin, 8 mg of sodium tetraborate decahydrate, 8 mg of bovine serum albumin and 0.8 mL of gelatin-phosphate buffer solution, pH 7.0 add water to make 8 mL, and lyophilize. Dissolve this in 8 mL of water. Prepare before use.

**Peroxidase-labeled rabbit anti-ECP antibody Fab' TS** Mix 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and then immunize rabbits subcutaneously in the back region and intramuscularly in the hind leg muscle with this solution 5 times at 2 week intervals. Harvest blood on the 10<sup>th</sup> day after completing the immunization to obtain rabbit antiserum. Rabbit anti-ECP antibody Fab' is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to agarose gel and then purifying by affinity column chromatography to obtain rabbit anti-ECP antibody which undergoes pepsin digestion to yield F(ab')<sub>2</sub> which is reacted with 2-aminoethanethiol hydrochloride.

Horseradish peroxidase is reacted with maleimido reagent [4-(maleimidomethyl) cyclohexyl carbonic acid-*N*-hydrox-

ysuccinimide imidoester] to yield maleimido peroxidase. Perform a coupling reaction by mixing rabbit anti-ECP antibody Fab' and maleimido peroxidase at 4°C to prepare peroxidase-labeled rabbit anti-ECP antibody Fab'. Take a specific amount of peroxidase-labeled rabbit anti-ECP antibody Fab' and dilute using PBS containing bovine serum albumin. The peroxidase-labeled rabbit anti-ECP antibody Fab' TS is a diluted solution with a concentration that gives a good calibration curve with assay characteristics.

**Description:** Clear and colorless solution

**Identification:** Pipet 100  $\mu$ L of the TS to be examined into flat-bottomed microtest plates. When substrate buffer solution for celmoleukin is added to this, it immediately exhibits a dark violet color, which changes to yellowish-red with time.

**Perphenazine maleate for assay**  $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$  [Same as the monograph Perphenazine Maleate. When dried, it contains not less than 99.0% of perphenazine maleate ( $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$ ).]

**Pethidine hydrochloride for assay**  $C_{15}H_{21}NO_2.HCl$  [Same as the monograph Pethidine Hydrochloride. When dried, it contains not less than 99.0% of pethidine hydrochloride  $C_{15}H_{21}NO_2.HCl$ .]

**Petrolatum** [Same as the monograph Yellow Petrolatum or White Petrolatum]

**Petroleum benzine** [K 8594, Special class]

**Petroleum ether** [K 8593, Special class]

**Phenacetin**  $C_{10}H_{13}NO_2$  White crystals or crystalline powder. Soluble in ethanol (95), and very slightly soluble in water.

**Melting point** <2.60>: 134 – 137°C

**Loss on drying** <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

***o*-Phenanthroline** See 1,10-phenanthroline monohydrate.

***o*-Phenanthroline hydrochloride** See 1,10-phenanthroline chloride monohydrate.

**1,10-Phenanthroline monohydrate**  $C_{12}H_8N_2.H_2O$  [K 8789, Special class]

***o*-Phenanthroline TS** See 1,10-phenanthroline TS.

**1,10-Phenanthroline TS** Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of a freshly prepared ferrous sulfate heptahydrate solution (37 in 2500) and 1 mL of dilute sulfuric acid. Preserve in tightly stoppered containers.

**1,10-Phenanthroline chloride monohydrate**  $C_{12}H_8N_2.HCl.H_2O$  [K 8202, Special class]

**Phenethylamine hydrochloride**  $C_6H_5CH_2CH_2NH_2.HCl$  White crystals or crystalline powder.

**Melting point:** <2.60> 220 – 225°C

**Phenobarbital for assay**  $C_{12}H_{12}N_2O_3$  [Same as the monograph Phenobarbital]

**Phenol**  $C_6H_5OH$  [K 8798, Special class]

**Phenol for assay**  $C_6H_5OH$  [K 8798, Phenol, Special class]

**Phenol-hydrochloric acid TS** Dissolve 0.2 g of phenol in

10 mL of 6 mol/L hydrochloric acid TS.

**Phenolphthalein**  $C_{20}H_{14}O_4$  [K 8799, 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 phenolphthalein in 100 mL of ethanol (95).

**Phenolphthalein TS, alkaline** See Alcohol Number Determination <1.01>.

**Phenol red**  $C_{19}H_{14}O_5S$  [K 8800, Special class]

**Phenol red TS** Dissolve 0.1 g of phenol red in 100 mL 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.

**Phenol-sodium nitroprusside TS** See phenol-sodium pentacyanonitrosylferrate (III) TS.

**Phenol-sodium pentacyanonitrosylferrate (III) TS** Dissolve 5 g of phenol and 25 mg of sodium pentacyanonitrosylferrate (III) dihydrate in sufficient water to make 500 mL. Preserve in a dark, cold place.

**Phenolsulfonphthalein for assay**  $C_{19}H_{14}O_5S$  [Same as the monograph Phenolsulfonphthalein. When dried, it contains not less than 99.0% of phenolsulfonphthalein ( $C_{19}H_{14}O_5S$ ).]

**Phenylalanine** See L-phenylalanine.

**L-Phenylalanine**  $C_9H_{11}NO_2$  [Same as the namesake monograph]

**Phenyl benzoate**  $C_6H_5COOC_6H_5$  White crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 68 – 70°C

*Purity* Clarity of solution—Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol: the solution is clear.

**25% Phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography** Prepared for gas chromatography.

**o-Phenylenediamine**  $H_2NC_6H_4NH_2$  White to dark brown crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in water.

*Content*: not less than 95.0%. Assay—Accurately weigh about 0.15 g of o-phenylenediamine, add 50 mL of acetic acid for nonaqueous titration to dissolve, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Correct by conducting a blank test using the same method.

Each mL of 0.1 mol/L perchloric acid VS  
= 10.81 mg of  $H_2NC_6H_4NH_2$

**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 titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 9.053 mg of  $H_2NC_6H_4NH_2 \cdot 2HCl$

**Phenylfluorone**  $C_{19}H_{12}O_5$  [K 9547]

**Phenylfluorone-ethanol TS** Dissolve 50 mg of phenylfluorone in 10 mL of a mixture of ethanol (95) and diluted hydrochloric acid (1 in 3), and add ethanol (95) to make exactly 500 mL.

**D-Phenylglycine**  $C_8H_9NO_2$  White, crystals or crystalline powder. Slightly soluble in water.

*Loss on drying* <2.41>: 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 <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 15.12 mg of  $C_8H_9NO_2$

**Phenylhydrazine**  $C_6H_5NHNH_2$  Colorless or light yellow, clear liquid, having a faint aromatic odor.

*Content*: not less than 99.0%. Assay—Weigh accurately about 1 g, add 30 mL of diluted hydrochloric acid (1 in 100) and water to make exactly 100 mL. Put exactly 20 mL of this solution in a glass-stoppered conical flask, and add 40 mL of diluted hydrochloric acid (3 in 4). After cooling, add 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking vigorously until the red color of the chloroform layer disappears. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L potassium iodate VS  
= 5.407 mg of  $C_6H_5NHNH_2$

**Phenylhydrazine hydrochloride** See phenylhydrazinium chloride.

**Phenylhydrazine hydrochloride TS** See phenylhydrazinium chloride TS.

**Phenylhydrazinium chloride**  $C_6H_5NHNH_2 \cdot HCl$  [K 8203, Special class]

**Phenylhydrazinium chloride TS** Dissolve 65 mg 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.

**Phenyl isothiocyanate**  $C_7H_5NS$  Prepared for amino acid analysis or biochemistry.

**1-phenyl-3-methyl-5-pyrazolone** See 3-methyl-1-phenyl-5-pyrazolone.

**50% Phenyl-50% methylpolysiloxane for gas chromatography** Prepared for gas 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.

**Phenylpiperazine hydrochloride** See phenylpiperazine monohydrochloride.

**Phenylpiperazine monohydrochloride**  $C_{10}H_{14}N_2 \cdot HCl$  A white powder. Melting point: about 247°C (with decomposition).

**Phenytoin for assay**  $C_{15}H_{12}N_2O_2$  [Same as the monograph Phenytoin. It meets the following requirements.]

**Purity** Related substances—Dissolve 25 mg of phenytoin for assay in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than phenytoin obtained from the sample solution is not larger than the peak area of phenytoin from the standard solution.

**Operating conditions**

**Detector:** An ultraviolet absorption photometer (wavelength: 220 nm).

**Column, column temperature, and flow rate:** Proceed as directed in the operating conditions in the Assay under Phenytoin.

**Mobile phase:** A mixture of 0.02 mol/L phosphate buffer solution, pH 3.5 and acetonitrile for liquid chromatography (11:9).

**Time span of measurement:** About 5 times as long as the retention time of phenytoin, beginning after the solvent peak.

**System suitability**

**Test for required detectability:** To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of phenytoin obtained from 10  $\mu$ L of this solution is equivalent to 8 to 12% of that with 10  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phenytoin are not less than 6000 and not more than 2.0, respectively.

**System repeatability:** When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phenytoin is not more than 2.0%.

**Phloroglucin** See phloroglucinol dihydrate.

**Phloroglucin dihydrate** See phloroglucinol dihydrate.

**Phloroglucinol dihydrate**  $C_6H_3(OH)_3 \cdot 2H_2O$  White to pale yellow, crystals or crystalline powder.

**Melting point** <2.60>: 215 – 219°C (after drying).

**Loss on drying** <2.41>: 18.0 – 24.0% (1 g, 105°C, 1 hour).

**Phosphatase, alkaline** 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  $\mu$ mol of 4-nitrophenol at 37°C and pH 9.8 in 1 minute from 4-nitrophenyl phosphate used as the substrate.

**Phosphatase TS, alkaline** Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

**Phosphate-buffered sodium chloride TS** Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate, and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.01 mol/L Phosphate buffer-sodium chloride TS, pH 7.4** Dissolve 2.93 g of disodium hydrogen phosphate dodecahydrate ( $NaH_2PO_4 \cdot 12H_2O$ ), 0.25 g of potassium dihydrogen phosphate ( $KH_2PO_4$ ), and 9.0 g of sodium chloride in water to make 1000 mL.

**Phosphate buffer solution for assay of bupleurum root** To 100 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 59 mL of sodium hydrogen phosphate TS.

**Phosphate buffer solution for component determination of bupleurum root** See phosphate buffer solution for assay of bupleurum root.

**Phosphate buffer solution for microplate washing** Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate dodecahydrate, 52.6 g of sodium chloride, 3.0 g of polysorbate 80 and 1.8 g of polyoxyethylene (40) octylphenyl ether in water to make 600 mL. Dilute this solution 10 times with water before use.

**Phosphate buffer solution for pancreatin** Dissolve 3.3 g of anhydrous disodium hydrogen phosphate, 1.4 g of potassium dihydrogen phosphate and 0.33 g of sodium chloride in water to make 100 mL.

**Phosphate buffer solution for processed aconite root** Dissolve 19.3 g of disodium hydrogen phosphate dodecahydrate in 3660 mL of water, and add 12.7 g of phosphoric acid.

**Phosphate buffer solution, pH 3.0** Dissolve 136 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.0 with phosphoric acid.

**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).

**Phosphate buffer solution, pH 3.1** Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water, and add 6.3 mL of phosphoric acid and water to make 1000 mL.

**0.02 mol/L Phosphate buffer solution, pH 3.5** Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10).

**0.05 mol/L Phosphate buffer solution, pH 3.5** To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of a solution of phosphoric acid (49 in

10,000) to make a solution having pH 3.5.

**0.1 mol/L Phosphate buffer solution, pH 4.5** Dissolve 13.61 g of potassium dihydrogen phosphate in 750 mL of water, adjust to pH 4.5 with potassium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution, pH 5.3** Dissolve 0.44 g of disodium hydrogen phosphate dodecahydrate and 13.32 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 5.3 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL.

**1/15 mol/L Phosphate buffer solution, pH 5.6** Dissolve 9.07 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 5.6 with potassium hydroxide TS, and add water to make 1000 mL.

**Phosphate buffer solution, pH 5.9** Dissolve 6.8 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 5.9 with diluted potassium hydroxide TS (1 in 10), and add water to make 1000 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.

**0.05 mol/L Phosphate buffer solution, pH 6.0** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 5.70 mL of 0.2 mol/L sodium hydroxide TS and water to make 200 mL.

**Phosphate buffer solution, pH 6.2** Dissolve 9.08 g of potassium dihydrogen phosphate in 1000 mL of water (solution A). Dissolve 9.46 g of disodium hydrogen phosphate in 1000 mL of water (solution B). Mix 800 mL of the solution A and 200 mL of the solution B, and adjust the pH to 6.2 with the solution A or the solution B if necessary.

**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 for antibiotics, pH 6.5** Dissolve 10.5 g of disodium hydrogen phosphate dodecahydrate and 5.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.5 with sodium hydroxide TS, and add water to make 1000 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.

**0.01 mol/L Phosphate buffer solution, pH 6.8** Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 6.8 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution, pH 6.8** Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate dodecahydrate in about 750 mL of water, adjust the pH to 6.8 with sodium hydroxide TS if necessary, and add 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 solu-

tion 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 dodecahydrate 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 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide 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.

**0.03 mol/L Phosphate buffer solution, pH 7.5** Dissolve 4.083 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 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.

**0.02 mol/L Phosphate buffer solution, pH 8.0** To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 300 mL of water, adjust the pH to 8.0 with sodium hydroxide TS, and add water to make 500 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 750 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL.

**0.1 mol/L Phosphate buffer solution for antibiotics, pH 8.0** Dissolve 16.73 g of dipotassium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 8.0 with phosphoric acid, and add water to make 1000 mL.

**0.2 mol/L Phosphate buffer solution, pH 10.5** Dissolve 34.8 g of dipotassium hydrogen phosphate in 750 mL of water, adjust to pH 10.5 with 8 mol/L sodium hydroxide TS, 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.

**Phosphinic acid**  $\text{H}_3\text{PO}_2$  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 1 mL of phosphinic acid add the mixture of iodine TS (1 mL) and water (20 mL): the iodine color disappears.

**Content:** 30.0 – 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 <2.50> liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L bromine VS  
= 1.650 mg of H<sub>3</sub>PO<sub>2</sub>

**Phosphate TS** Dissolve 2.0 g of dipotassium hydrogen phosphate and 8.0 g of potassium dihydrogen phosphate in water to make 1000 mL.

**Phosphomolybdic acid** See phosphomolybdic acid *n*-hydrate.

**Phosphomolybdic acid *n*-hydrate** P<sub>2</sub>O<sub>5</sub>·24MoO<sub>3</sub>·*x*H<sub>2</sub>O  
Yellow crystals or crystalline powder.

**Identification** (1) To 10 mL of a solution (1 in 10) add 0.5 mL of ammonia TS: yellow precipitates appear, which disappear by the addition of 2 mL of ammonia TS, and yellow precipitates appear by further addition of 5 mL of diluted nitric acid (1 in 2).

(2) To 5 mL of a solution (1 in 10) add 1 mL of ammonia TS and 1 mL of magnesia TS: white precipitates appear.

**Phosphoric acid** H<sub>3</sub>PO<sub>4</sub> [K 9005, Special class]

**Phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0** Dissolve 6.77 mL of phosphoric acid, 5.72 mL of acetic acid (100) and 6.18 g of boric acid in water to make 1000 mL. Adjust the pH of this solution to 2.0 with 0.5 mol/L sodium hydroxide VS.

**Phosphoric acid-sodium sulfate buffer solution, pH 2.3** Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, and add 2.7 mL of phosphoric acid. If necessary, adjust to pH 2.3 with 2-aminoethanol.

**Phosphorus pentoxide** See phosphorus (V) oxide.

**Phosphorus, red** P An odorless dark red powder. Practically insoluble in carbon disulfide and in water.

**Free phosphoric acid:** Not more than 0.5%.

To 5 g add 10 mL of a solution of sodium chloride (1 in 5), mix, then add 50 mL of the solution of sodium chloride (1 in 5), allow to stand for 1 hour, and filter. Wash the residue with three 10-mL portions of the solution of sodium chloride (1 in 5), combine the filtrate and the washings, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of thymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 4.90 mg of H<sub>3</sub>PO<sub>4</sub>

**Phosphorus (V) oxide** P<sub>2</sub>O<sub>5</sub> [K 8342, Special class]

**Phosphotungstic acid** See phosphotungstic acid *n*-hydrate.

**Phosphotungstic acid *n*-hydrate** P<sub>2</sub>O<sub>5</sub>·24WO<sub>3</sub>·*x*H<sub>2</sub>O  
White to yellowish green, crystals or crystalline powder.

**Identification**—To 5 mL of a solution (1 in 10) add 1 mL of acidic tin (II) chloride TS, and heat: blue precipitates appear.

**Phosphotungstic acid TS** Dissolve 1 g of phosphotungstic acid *n*-hydrate in water to make 100 mL.

***o*-Phthalaldehyde** C<sub>6</sub>H<sub>4</sub>(CHO)<sub>2</sub> Light yellow to yellow crystals.

**Content:** not less than 99%. **Assay**—Dissolve 1 g of *o*-phthalaldehyde in 10 mL of ethanol (95). Proceed with 2 μL of this solution as directed in Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method.

**Content (%) =** peak area of *o*-phthalaldehyde/total area of all peaks × 100

**Operating conditions**

**Detector:** A thermal conductivity detector.

**Column:** A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography treated with acid and silane (177 – 250 μm), coated with methyl silicon polymer for gas chromatography in 10%.

**Column temperature:** A constant temperature of about 180°C.

**Carrier gas:** Helium.

**Flow rate:** Adjust the flow rate so that the retention time of *o*-phthalaldehyde is 3 – 4 minutes.

**Time span of measurement:** About 7 times as long as the retention time of *o*-phthalaldehyde, beginning after the solvent peak.

**Phthalein purple** C<sub>32</sub>H<sub>32</sub>N<sub>2</sub>O<sub>12</sub>·*x*H<sub>2</sub>O Yellowish white to brown powder. Soluble in ethanol (95), and practically insoluble in water.

**Sensitivity test**—Dissolve 10 mg of phthalein purple in 1 mL of ammonia solution (28), and add water to make 100 mL. To 5 mL of this solution add 95 mL of water, 4 mL of ammonia solution (28), 50 mL of ethanol (95) and 0.1 mL of diluted barium chloride TS (1 in 5): the solution shows a blue-purple color which disappears on the addition of 0.15 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

**Phthalic acid** C<sub>8</sub>H<sub>6</sub>O<sub>4</sub> Colorless or white crystalline powder. Soluble in methanol and in ethanol (95), sparingly soluble in water, and practically insoluble in chloroform. Melting point: about 200°C (with decomposition).

**Content:** not less than 98%. **Assay**—Weigh accurately about 2.8 g of phthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide VS and 25 mL of water, and dissolve by heating on a hot plate. After cooling, add 5 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS. Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS  
= 83.07 mg of C<sub>8</sub>H<sub>6</sub>O<sub>4</sub>

**Phthalic anhydride** C<sub>8</sub>H<sub>4</sub>O<sub>3</sub> White crystals or crystalline powder.

**Melting point** <2.60>: 131 – 134°C.

**Phthalimide**  $C_8H_5NO_2$  White to pale brown crystals or powder.

*Melting point* <2.60>: 232 – 237°C

*Clarity*—1.0 g of phthalimide dissolves in 20 mL of sodium hydroxide TS as a slight turbid solution.

*Content*: not less than 98.0%. *Assay*—Weigh accurately about 0.3 g of the substance to be tested, dissolve in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS  
= 14.71 mg of  $C_8H_5NO_2$

**Phytonadione**  $C_{31}H_{46}O_2$  [Same as the namesake monograph]

**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.

**Pig bile powder for thin-layer chromatography** A yellow-gray to yellow-brown powder, having a characteristic odor and a bitter taste. It is practically insoluble in water, in methanol and in ethanol (99.5).

*Identification*—To 0.1 g of pig bile powder for thin-layer chromatography in a screw-capped test tube, add 1 mL of sodium hydroxide solution (3 in 25), and shake. Heat the tube in an oil bath at 120°C for 4 hours, allow to cool to a lukewarm temperature, add 2 mL of 3 mol/L hydrochloric acid TS and 2 mL of ethyl acetate, shake at 50°C for 30 minutes, and separate ethyl acetate layer as the sample solution. Separately, dissolve 10 mg of hyodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: one of the several spots obtained from the sample solution shows the same color tone and the same *Rf* value as the spot from the standard solution.

**Piperidine hydrochloride**  $C_5H_{11}N.HCl$  A white crystalline powder. Dissolves in water and in methanol. The pH of the aqueous solution (1 in 20) is between 3.0 and 5.0.

*Melting point* <2.60>: 247 – 252°C

*Purity* *Clarity and color of solution*—Dissolve 1.0 g of piperidine hydrochloride in 20 mL of water: the solution is clear and colorless.

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Content*: not less than 99.0%. *Assay*—Dissolve about 0.25 g of piperidine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 12.16 mg of  $C_5H_{11}N.HCl$

**Plantago seed for thin-layer chromatography** [Same as the monograph Plantago Seed meeting the following additional specifications.]

*Identification* (1) To 1 g of pulverized plantago seed for thin-layer chromatography add 3 mL of methanol, and warm on a water bath for 3 minutes. After cooling, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 10 minutes: spots equivalent to those described below appear.

<i>Rf</i> value	Color and shape of the spot
Around 0	A strong spot, very dark blue
Around 0.08	A very dark blue spot
Around 0.1 – 0.2	A leading spot, very dark blue
Around 0.25	A strong spot, deep blue (corresponding to plantagouanidinic acid)
Around 0.35	A strong spot, dark grayish blue (corresponding to geniposidic acid)
Around 0.45	A weak spot, grayish yellowish green
Around 0.50	A strong spot, deep yellow-green (corresponding to acteoside)
Around 0.6	A weak spot, light blue
Around 0.85	A deep blue spot
Around 0.9 – 0.95	A tailing spot, grayish blue

(2) Proceed as directed in the operating conditions under (1), except using a mixture of ethyl acetate, water and formic acid (6:1:1) as developing solvent: spots equivalent to those described below appear.

<i>Rf</i> value	Color and shape of the spot
Around 0	A yellow-greenish dark gray spot
Around 0.05	A weak spot, dark grayish yellow-green
Around 0.2	A weak spot, dark green
Around 0.25	A strong spot, dark reddish purple (corresponding to geniposidic acid)
Around 0.35	A weak spot, bright blue
Around 0.4 – 0.45	A weak tailing spot, dull greenish blue
Around 0.45	A strong spot, deep yellow-green (corresponding to acteoside)
Around 0.5	A strong spot, deep blue (corresponding to plantagouanidinic acid)
Around 0.95	A strong spot, dark grayish blue-green
Around 0.97	A dark grayish blue-green spot

**Polyalkylene glycol for gas chromatography** Prepared for gas chromatography.

**Polyalkylene glycol monoether for gas chromatography**  
Prepared for gas chromatography.

**Polyethylene glycol 15000-diepoxyde for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol ester 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 600 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 1500 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 6000 for gas chromatography** Prepared for gas chromatography.

**Polyethylene glycol 2-nitroterephthalate for gas chromatography** Prepared for gas chromatography.

**Polymethyl acrylate for gas chromatography** Prepared for gas chromatography.

**Polymethylsiloxane for gas chromatography** Prepared for gas chromatography.

**Polyoxyethylene hydrogenated castor oil 60** A nonionic surfactant prepared by addition polymerization of ethylene oxide with 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 and in 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 10 mL of water and 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, and shake thoroughly. Add 5 mL 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 10 mL of water, shake, and add 5 drops of bromine TS: the color of the test solution does not disappear.

*Congealing point* <2.42>: 30 – 34°C

*pH* <2.54>—To 1.0 g of polyoxyethylene hydrogenated castor oil 60 add 20 mL of water, and dissolve by heating: the pH of the solution is between 3.6 and 6.0.

*Acid value* <1.13>: not more than 1.0.

*Saponification value* <1.13>: 41 – 51

*Hydroxyl value* <1.13>: 39 – 49

*Purity* (1) Clarity and color of solution—Dissolve 1.0 g of polyoxyethylene hydrogenated castor oil 60 in 20 mL of ethanol: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of polyoxyethylene hydrogenated castor oil 60 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g

of polyoxyethylene hydrogenated castor oil 60 according to Method 3, and perform the test (not more than 2 ppm).

*Water* <2.48>: not more than 2.0% (1 g).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Storage*—Preserve in tight containers.

#### **Polyoxyethylene (23) lauryl ether**

$C_{12}H_{25}(OCH_2CH_2)_nOH$  White masses. Melting point: about 40°C

**Polyoxyethylene (40) octylphenyl ether** Obtained by the addition polymerization with ethylene oxide to octylphenol. A colorless or white to pale yellow, liquid, vaseline-like or waxy, having slightly a characteristic odor.

*pH* <2.54>: 7.0 – 9.5 (5 w/v%, 25°C).

*Specific gravity* <2.56>  $d_4^{20}$ : 1.10 – 1.11

*Purity* Clarity—a solution (1 in 20) is clear.

**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 10 mL of water and 10 mL of sodium hydroxide TS, boil for 5 minutes, and acidify with dilute hydrochloric acid: an oily fraction is separated.

(2) To 0.5 g of polysorbate 20 add 10 mL of water, shake, 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 Saponification Value, and evaporate ethanol completely. Dissolve the residue in 50 mL of water, acidity with hydrochloric acid (methyl orange), and extract with two 30 mL portions of diethyl ether. Combine the diethyl ether layer, wash with 20 mL portions 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 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS for saponification.

*Acid value* <1.13>: not more than 4.0.

*Saponification value* <1.13>: 43 – 55

*Loss on drying* <2.41>: 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 15 mL 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].

**Polyvinyl alcohol**  $(-CH_2CHOH-)_n$  [K 9550, Special class]

**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 in diethyl ether. To polyvinyl alcohol I add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

**Viscosity <2.53>**: 25.0 – 31.0 mm<sup>2</sup>/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a 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.

**pH <2.54>**—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 20 mL 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**: 98.0 – 99.0 mol%. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and dissolve by heating on a water bath. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake well, and titrate <2.50> 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 25 mL or more, use about 2.0 g of the sample.

Saponification value (mol%)

$$= 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{0.6005 \times (a - b)f}{\text{amount (g) of the sample}}$$

*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 in diethyl ether. To polyvinyl alcohol II add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

**Viscosity <2.53>**: 4.6 – 5.4 mm<sup>2</sup>/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL 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.

**pH <2.54>**—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 20 mL of water, disperse by well stirring, heat on a water bath for 2 hours, and cool: the solution is clear and colorless.

**Saponification value**: 86.5 – 89.5 mol%. Weigh accurately about 2.0 g of polyvinyl alcohol II, previously dried, transfer to a glass-stoppered, conical flask, add 100 mL of water, and warm while stirring for 2 hours. After cooling, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, stopper tightly,

and allow to stand for 2 hours. Then add exactly 30 mL of 0.25 mol/L sulfuric acid VS, shake well, and titrate <2.50> 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.

Saponification value (mol%)

$$= 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a - b)f}{\text{amount (g) of the sample}}$$

*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 100 mL.

**Potassium acetate** CH<sub>3</sub>COOK [K 8363, Special class]

**Potassium acetate TS** Dissolve 10 g of potassium acetate in water to make 100 mL (1 mol/L).

**Potassium aluminum sulfate** See aluminum potassium sulfate dodecahydrate.

**Potassium bicarbonate** See potassium hydrogen carbonate.

**Potassium biphthalate** See potassium hydrogen phthalate.

**Potassium biphthalate buffer solution, pH 3.5** See potassium hydrogen phthalate buffer solution, pH 3.5.

**Potassium biphthalate buffer solution, pH 4.6** See potassium hydrogen phthalate buffer solution, pH 4.6.

**Potassium biphthalate buffer solution, pH 5.6** See potassium hydrogen phthalate buffer solution, pH 5.6.

**Potassium biphthalate for pH determination** See potassium hydrogen phthalate for pH determination.

**Potassium biphthalate (standard reagent)** See potassium hydrogen phthalate (standard reagent).

**0.2 mol/L Potassium biphthalate TS for buffer solution** See 0.2 mol/L potassium hydrogen phthalate TS for buffer solution.

**Potassium bisulfate** See potassium hydrogen sulfate.

**Potassium bromate** KBrO<sub>3</sub> [K 8530, Special class]

**Potassium bromide** KBr [K 8506, Special class]

**Potassium 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 <2.25>: any abnormal absorption does not appear.

**Potassium carbonate** K<sub>2</sub>CO<sub>3</sub> [K 8615, 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 100 mL.

**Potassium chlorate**  $\text{KClO}_3$  [K 8207, Special class]

**Potassium chloride**  $\text{KCl}$  [K 8121, Special class]

**Potassium chloride for conductivity measurement** [K 8121, Potassium chloride for conductivity measurement]

**Potassium chloride for infrared spectrophotometry**  
Crush homocrystals of potassium chloride or potassium chloride (Special class), collect the powder passed through a No. 200 (75  $\mu\text{m}$ ) 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 <2.25>: any abnormal absorption does not appear.

**Potassium chloride-hydrochloric acid buffer solution** To 250 mL of a solution of potassium chloride (3 in 20) add 53 mL of 2 mol/L hydrochloric acid TS and water to make 1000 mL.

**Potassium chloride TS, acidic** Dissolve 250 g of potassium chloride in water to make 1000 mL, and add 8.5 mL of hydrochloric acid.

**0.2 mol/L Potassium chloride TS** Dissolve 14.9 g of potassium chloride in water to make 1000 mL. Prepare before use.

**Potassium chromate**  $\text{K}_2\text{CrO}_4$  [K 8312, Special class]

**Potassium chromate TS** Dissolve 10 g of potassium chromate in water to make 100 mL.

**Potassium cyanide**  $\text{KCN}$  [K 8443, Special class]

**Potassium cyanide TS** Dissolve 1 g of potassium cyanide in water to make 10 mL. Prepare before use.

**Potassium dichromate**  $\text{K}_2\text{Cr}_2\text{O}_7$  [K 8517, Special class]

**Potassium dichromate (standard reagent)**  $\text{K}_2\text{Cr}_2\text{O}_7$  [K 8005, 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 100 mL.

**Potassium dichromate TS** Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

**Potassium dihydrogen phosphate**  $\text{KH}_2\text{PO}_4$  [K 9007, Special class]

**Potassium dihydrogen phosphate for pH determination**  $\text{KH}_2\text{PO}_4$  [K 9007, for pH determination]

**0.1 mol/L Potassium dihydrogen phosphate TS, pH 2.0**  
Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 2.0 with phosphoric acid.

**0.25 mol/L Potassium dihydrogen phosphate TS, pH 3.5**  
Dissolve 34 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

**0.05 mol/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.

**0.05 mol/L Potassium dihydrogen phosphate TS, pH 4.7**  
Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to exactly 4.7 with dilute sodium hydrochloride TS, and add water to make 1000 mL.

**0.01 mol/L Potassium dihydrogen phosphate TS, pH 4.0**  
Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

**0.02 mol/L Potassium dihydrogen phosphate TS** Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.05 mol/L Potassium dihydrogen phosphate TS** Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.33 mol/L Potassium dihydrogen phosphate TS** Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 100 mL.

**0.1 mol/L Potassium dihydrogen phosphate TS** Dissolve 13.61 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.2 mol/L Potassium dihydrogen phosphate TS** Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

**0.2 mol/L Potassium dihydrogen phosphate TS for buffer solution** Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000 mL.

**Potassium disulfate**  $\text{K}_2\text{S}_2\text{O}_7$  [K 8783, Potassium Disulfate, Special class]

**Potassium ferricyanide** See potassium hexacyanoferrate (III).

**Potassium ferricyanide TS** See potassium hexacyanoferrate (III) TS.

**Potassium ferricyanide TS, alkaline** See potassium hexacyanoferrate (III) TS, alkaline.

**Potassium ferrocyanide** See potassium hexacyanoferrate (II) trihydrate.

**Potassium ferrocyanide TS** See potassium hexacyanoferrate (II) TS.

**Potassium guaiacolsulfonate**  $\text{C}_7\text{H}_7\text{KO}_5\text{S}$  [Same as the namesake monograph]

**Potassium hexacyanoferrate (II) trihydrate**  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  [K 8802, Special class]

**Potassium hexacyanoferrate (II) TS** Dissolve 1 g of potassium hexacyanoferrate (II) trihydrate in water to make 10 mL (1/4 mol/L). Prepare before use.

**Potassium hexacyanoferrate (III)**  $\text{K}_3\text{Fe}(\text{CN})_6$  [K 8801, Special class]

**Potassium hexacyanoferrate (III) TS** Dissolve 1 g of potassium hexacyanoferrate (III) in water to make 10 mL (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 100 mL. Preserve in light-resistant containers.

**Potassium hexahydroxoantimonate (V)**  $K_2H_2Sb_2O_7$   
White granules or crystalline powder.

**Identification**—To 1 g add 100 mL of water, and dissolve by warming. To 20 mL of this solution add 0.2 mL of sodium chloride TS: white precipitates appear. Rubbing the inside wall of the vessel with a glass rod accelerates the forming of the precipitates.

**Potassium hexahydroxoantimonate (V) TS** To 2 g of potassium hexahydroxoantimonate (V) add 100 mL of water. Boil the solution for about 5 minutes, cool quickly, add 10 mL of a solution of potassium hydroxide (3 in 20), allow to stand for 1 day, and filter.

**Potassium hydrogen carbonate**  $KHCO_3$  [K 8621, Special class]

**Potassium hydrogen phthalate**  $C_6H_4(COOK)(COOH)$  [K 8809, Special class]

**Potassium hydrogen phthalate buffer solution, pH 3.5**  
Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97 mL of 0.2 mol/L hydrochloric acid VS with water to make 200 mL.

**Potassium hydrogen phthalate buffer solution, pH 4.6**  
Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

**0.3 mol/L Potassium hydrogen phthalate buffer solution, pH 4.6** Dissolve 61.26 g of potassium hydrogen phthalate in about 800 mL of water, adjust the pH to 4.6 with sodium hydroxide TS, and add water to make 1000 mL.

**Potassium hydrogen phthalate buffer solution, pH 5.6**  
Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

**Potassium hydrogen phthalate for pH determination**  
 $C_6H_4(COOK)(COOH)$  [K 8809, For pH determination]

**Potassium hydrogen phthalate (standard reagent)**  
 $C_6H_4(COOK)(COOH)$  [K 8005, 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 1000 mL.

**Potassium hydrogen sulfate**  $KHSO_4$  [K 8972, Special class]

**Potassium hydroxide**  $KOH$  [K 8574, Special class]

**Potassium hydroxide-ethanol TS** Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100 mL. Prepare before use.

**0.1 mol/L Potassium hydroxide-ethanol TS** To 1 mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5 mL. Prepare before use.

**Potassium hydroxide-ethanol TS, dilute** Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol (95) to make 1000 mL (0.5 mol/L). Preserve in tightly stoppered bottles.

**Potassium hydroxide TS** Dissolve 6.5 g of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in

polyethylene bottles.

**0.02 mol/L Potassium hydroxide TS** Dilute 2 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

**0.05 mol/L Potassium hydroxide TS** Dilute 5 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

**8 mol/L Potassium hydroxide TS** Dissolve 52 g of potassium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

**Potassium iodate**  $KIO_3$  [K 8922, Special class]

**Potassium iodate (standard reagent)**  $KIO_3$  [K 8005, Standard reagent for volumetric analysis]

**Potassium iodide**  $KI$  [K 8913, Special class]

**Potassium iodide for assay** [Same as the monograph Potassium Iodide]

**Potassium iodide-starch TS** Dissolve 0.5 g of potassium iodide in 100 mL of freshly prepared starch TS. Prepare before use.

**Potassium iodide TS** Dissolve 16.5 g of potassium iodide in water to make 100 mL. Preserve in light-resistant containers. Prepare before use (1 mol/L).

**Potassium iodide TS, concentrated** Dissolve 30 g of potassium iodide in 70 mL of water. Prepare before use.

**Storage**—Preserve in light-resistant containers.

**Potassium iodide TS, saturated** Saturate 20 g of potassium iodide in 10 mL of freshly boiled and cooled water. Prepare before use.

**Potassium iodide-zinc sulfate TS** Dissolve 5 g of potassium iodide, 10 g of zinc sulfate, and 50 g of sodium chloride in water to make 200 mL.

**Potassium methanesulfonate**  $CH_3SO_3K$  White crystals or crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of potassium methanesulfonate in 20 mL of water: the solution is transparent and colorless.

**Content:** not less than 98.0%. **Assay**—Dissolve about 0.1 g of potassium methanesulfonate, accurately weighed, in 10 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric 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 sulfonate** See potassium 1,2-naphthoquinone-4-sulfonate.

**Potassium 1,2-naphthoquinone-4-sulfonate**  $C_{10}H_5O_2SO_3K$  [K 8696,  $\beta$ -Naphthoquinone-4-sulfonic acid potassium salt, Special class]

**Potassium 1,2-naphthoquinone-4-sulfonate TS** Dissolve 0.5 g of potassium 1,2-naphthoquinone-4-sulfonate in water to make 100 mL. Prepare before use.

**Potassium nitrate**  $KNO_3$  [K 8548, Special class]

**Potassium nitrite**  $\text{KNO}_2$  A white to pale yellow crystalline powder. It is deliquescent.

*Identification*—(1) Dissolve 1 g of potassium nitrite in 20 mL of water, and use this as the sample solution. To 5 mL of the sample solution add 1 mL of sulfuric acid: a yellowish brown gas is evolved.

(2) The sample solution obtained in (1) responds to the Qualitative Tests <1.09> (1) for potassium salt.

Preserve in a light-resistant tight container.

**Potassium periodate**  $\text{KIO}_4$  [K 8249, Special class]

**Potassium periodate TS** To 2.8 g of potassium periodate add 200 mL of water, dissolve by adding dropwise 20 mL of sulfuric acid under shaking, cool, and add water to make 1000 mL.

**Potassium permanganate**  $\text{KMnO}_4$  [K 8247, Special class]

**Potassium permanganate TS** Dissolve 3.3 g of potassium permanganate in water to make 1000 mL (0.02 mol/L).

**Potassium permanganate TS, acidic** To 100 mL of potassium permanganate TS add 0.3 mL of sulfuric acid.

**Potassium peroxodisulfate**  $\text{K}_2\text{S}_2\text{O}_8$  [K 8253, Special class]

**Potassium persulfate** See potassium peroxodisulfate.

**Potassium pyroantimonate** See potassium hexahydroxantimonate (V).

**Potassium pyroantimonate TS** See potassium hexahydroxantimonate (V) TS.

**Potassium pyrophosphate**  $\text{K}_4\text{O}_7\text{P}_2$  White, crystalline powder, very soluble in water.

*Melting point* <2.60>: 1109°C

**Potassium pyrosulfate** See potassium disulfate.

**Potassium sodium tartarate** See potassium sodium tartarate tetrahydrate.

**Potassium sodium tartarate tetrahydrate**  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  [K 8536, (+)-Potassium sodium tartarate tetrahydrate, Special class]

**Potassium sulfate**  $\text{K}_2\text{SO}_4$  [K 8962, Special class]

**Potassium sulfate TS** Dissolve 1 g of potassium sulfate in water to make 100 mL.

**Potassium tartrate**  $2\text{C}_4\text{H}_4\text{K}_2\text{O}_6 \cdot \text{H}_2\text{O}$  [K 8535, (+)-Potassium Tartrate-Water (2/1), Special class]

**Potassium tellurite**  $\text{K}_2\text{TeO}_3$  White powder or small masses obtained by melting an equimolar mixture of tellurium dioxide and potassium carbonate in a stream of carbon dioxide. Soluble in water.

*Content*: not less than 90.0%. *Assay*—Dissolve about 1.0 g of potassium tellurite, accurately weighed, in 100 mL of water, add 5 mL of diluted acetic acid (31) (1 in 3), and boil. After cooling, filter by suction through a crucible glass filter (1G4), previously dried at  $105 \pm 2^\circ\text{C}$  for 1 hour to constant mass ( $b$  (g)). Wash the filtrate with water, dry the glass filter at  $110^\circ\text{C}$  for 3 hours, and measure the mass  $a$  (g).

Content (%) of potassium tellurite ( $\text{K}_2\text{TeO}_3$ )

$$= \frac{(a - b) \times 1.5902}{S} \times 100$$

S: Mass (g) of potassium tellurite taken.

**Potassium tetraoxalate for pH determination** See potassium trihydrogen dioxalate dihydrate for pH determination.

**Potassium thiocyanate**  $\text{KSCN}$  [K 9001, Special class]

**Potassium thiocyanate TS** Dissolve 1 g of potassium thiocyanate in water to make 10 mL.

**Potassium trihydrogen dioxalate dihydrate for pH determination**  $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$  [K 8474]

**Potato extract** Prepared for microbial test.

**Potato starch** [Same as the namesake monograph]

**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  $105^\circ\text{C}$  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 20 mL of water, and make it pasty by gradually adding 5 mL of a solution of sodium hydroxide (2 in 25) while shaking well. Heat in a water bath for 3 minutes while shaking, add 25 mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid TS, add 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0, and add water to make exactly 100 mL. Prepare before use.

**Powdered tragacanth** [Same as the namesake monograph]

**(±)-Praeruptorin A for thin-layer chromatography**  $\text{C}_{21}\text{H}_{22}\text{O}_7$  White crystals or crystalline powder. Soluble in methanol, sparingly slightly soluble in ethanol (99.5), and practically insoluble in water.

*Identification*—Determine the absorption spectrum of a solution of (±)-praeruptorin A for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 320 nm and 324 nm.

*Melting point* <2.60>: 152 – 156°C

*Purity* Related substances—Dissolve 2 mg of (±)-praeruptorin A for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5  $\mu\text{L}$  each of these solutions as directed in the Identification (1) under Peucedanum Root: the spot other than the principal spot of around  $R_f$  value of 0.3 from the sample solution is not more intense than the spot from the standard solution.

**Pravastatin sodium**  $\text{C}_{23}\text{H}_{35}\text{NaO}_7$  [Same as the namesake monograph]

**Prazepam for assay**  $\text{C}_{19}\text{H}_{17}\text{ClN}_2\text{O}$  [Same as the monograph Prazepam. When dried, it contains not less than 99.0% of  $\text{C}_{19}\text{H}_{17}\text{ClN}_2\text{O}$ .]

**Prednisolone**  $\text{C}_{21}\text{H}_{28}\text{O}_5$  [Same as the namesake mono-

graph]

**Prednisolone acetate**  $C_{23}H_{30}O_6$  [Same as the namesake 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.

*Optical rotation* <2.49>  $[\alpha]_D^{20}$ : +167 – +175° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

*Loss on drying* <2.41>: not more than 1.0% (1 g, 105°C, 3 hours).

*Content*: 96.0 – 104.0%. Assay—Weigh accurately about 20 mg of prednisone, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, dilute with methanol to make exactly 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and read the absorbance  $A$  at the wavelength of maximum absorption at about 238 nm.

$$\text{Amount (mg) of } C_{21}H_{26}O_5 = \frac{A}{440} \times 20,000$$

**Probenecid**  $C_{13}H_{19}NO_4S$  [Same as the namesake monograph]

**Procainamide hydrochloride**  $C_{13}H_{21}N_3O.HCl$  [Same as the namesake monograph]

**Procainamide hydrochloride for assay**  $C_{13}H_{21}N_3O.HCl$  [Same as the monograph Procainamide Hydrochloride. When dried, it contains not less than 99.0% of procainamide hydrochloride ( $C_{13}H_{21}N_3O.HCl$ ).]

**Procaine hydrochloride**  $C_{13}H_{20}N_2O_2.HCl$  [Same as the namesake monograph]

**Procaine hydrochloride for assay** [Same as the monograph Procaine Hydrochloride]

**Procatamol hydrochloride** See procatamol hydrochloride hydrate.

**Procatamol hydrochloride hydrate**  $C_{16}H_{22}N_2O_3.HCl.1/2H_2O$  [Same as the namesake monograph]

**Progesterone**  $C_{21}H_{30}O_2$  [Same as the namesake monograph]

**L-Proline**  $C_5H_9NO_2$  [K 9107, Special class]

**Propafenone hydrochloride for assay**  $C_{21}H_{27}NO_3.HCl$  [Same as the monograph Propafenone Hydrochloride. When dried, it contains not less than 99.0% of propafenone hydrochloride ( $C_{21}H_{27}NO_3.HCl$ ). When perform the test as directed in the Purity (2), the total area of the peaks other than the peak of propafenone is not larger than 3 times the peak area of propafenone from the standard solution.]

**n-Propanol** See 1-propanol.

**1-Propanol**  $CH_3CH_2CH_2OH$  [K 8838, Special class]

**2-Propanol**  $(CH_3)_2CHOH$  [K 8839, Special class]

**2-Propanol for vitamin A assay**  $(CH_3)_2CHOH$  [K 8839, Special class] When the absorbances at 300 nm and between 320 nm and 350 nm are determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control, they are not more than 0.05 and not

more than 0.01, respectively. If necessary, purify by distillation.

**2-Propanol for liquid chromatography**  $(CH_3)_2CHOH$  Clear, colorless and volatile liquid, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. Boiling point: about 82°C.

*Refractive index* <2.45>  $n_D^{20}$ : 1.376 – 1.378

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.785 – 0.788

*Purity* (1) Ultraviolet absorbing substances—Perform the test with 2-propanol for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at 230 nm is not more than 0.2; at 250 nm, not more than 0.03; and between 280 nm and 400 nm, not more than 0.01.

(2) Peroxide—Mix 100 mL of water and 25 mL of dilute sulfuric acid, and add 25 mL 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 <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005%).

**Propanol, iso** See 2-propanol.

**Propranolol hydrochloride for assay**  $C_{16}H_{21}NO_2.HCl$  [Same as the monograph Propranolol Hydrochloride. When dried, it contains not less than 99.5% of propranolol hydrochloride ( $C_{16}H_{21}NO_2.HCl$ ).]

**Propanteline bromide**  $C_{23}H_{30}BrNO_3$  [Same as the namesake monograph]

**Propionic acid**  $CH_3CH_2COOH$  Colorless liquid.

*Purity*—Clarity and color of solution—Dissolve 1 g of propionic acid in 20 mL of ethanol (95): the solution is clear and colorless.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.998 – 1.004

*Distilling range* <2.57>: 139 – 143°C, not less than 95 vol%.

**Propylamine, iso**  $(CH_3)_2CHNH_2$  Colorless liquid, having a characteristic, amine-like odor. Miscible with water, with ethanol (95) and with diethyl ether.

*Refractive index* <2.45>  $n_D^{20}$ : 1.374 – 1.376

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.685 – 0.690

*Distilling range* <2.57>: 31 – 33°C, not less than 95 vol%.

**Propyl benzoate**  $C_6H_5COOC_3H_7$  Clear, colorless liquid, having a characteristic odor.

*Refractive index* <2.45>  $n_D^{20}$ : 1.498 – 1.503

*Specific gravity* <2.56>  $d_{20}^{20}$ : 1.022 – 1.027

**Propylene carbonate**  $C_4H_6O_3$  Colorless liquid.

*Boiling point* <2.57>: 240 – 242°C

*Water* <2.48>: less than 0.1%

**Propylene carbonate for water determination** See Water Determination <2.48>.

**Propylene glycol**  $CH_3CH(OH)CH_2OH$  [K 8837, Special class]

**Propylene glycol cefatrizine**  $C_{18}H_{18}N_6O_5S_2.C_3H_8O_2$  [Same as the namesake monograph]

**Propylether, iso**  $(CH_3)_2CHOCH(CH_3)_2$  Clear, colorless

liquid, having a characteristic odor. Not miscible with water.

*Refractive index* <2.45>  $n_D^{20}$ : 1.368 – 1.369

*Specific gravity* <2.56>  $d_4^{20}$ : 0.723 – 0.725

**Propyl parahydroxybenzoate**

$\text{HOC}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_3$  [Same as the namesake monograph]

**Propylthiouracil for assay**  $\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$  [Same as the monograph Propylthiouracil. When dried, it contains not less than 99.0% of propylthiouracil ( $\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$ ).]

**Prostaglandin A<sub>1</sub>**  $\text{C}_{20}\text{H}_{32}\text{O}_4$  White crystals or crystalline powder. Very soluble in ethanol (95) and in ethyl acetate, and very slightly soluble in water.

*Purity* Related substances—Dissolve 5 mg of prostaglandin A<sub>1</sub> in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> 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 A<sub>1</sub> from the sample solution is not larger than the peak area of prostaglandin A<sub>1</sub> from the standard solution. Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed the operating conditions in the Assay of Alprostadil Alfadex.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of prostaglandin A<sub>1</sub> obtained from 10  $\mu\text{L}$  of the standard solution is 5 to 10% of the full scale.

Time span of measurement: About twice as long as the retention time of prostaglandin A<sub>1</sub> beginning after the solvent peak.

**Protein digestive enzyme TS** A solution of lysyl endopeptidase in 0.05 mol/L tris buffer solution, pH 8.6 (1 in 5000).

**Pseudoephedrine hydrochloride**  $\text{C}_{10}\text{H}_{15}\text{NO}\cdot\text{HCl}$  White, crystals or crystalline powder. Freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (99.5), and practically insoluble in acetic anhydride. Melting point: 182 – 186°C

*Purity* Related substances—Dissolve 1 mg in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10  $\mu\text{L}$  of the sample solution for twice as long as as the retention time of ephedrine as directed in the Assay (1) under Kakkonto Extract: the total area of the peaks other than pseudoephedrine and the solvent is not larger than 1/10 times the total area of the peaks other than the solvent.

**Puerarin for thin-layer chromatography**  $\text{C}_{21}\text{H}_{20}\text{O}_9$  White crystalline powder. Freely soluble in methanol, and practically insoluble in diethyl ether. Melting point: about 188°C (with decomposition).

*Purity* Related substances—Dissolve 1.0 mg of puerarin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 2  $\mu\text{L}$  of this solution as directed in the Identification under Pueraria Root: any spot other than the principal spot at the  $R_f$  value of about 0.4 does not appear.

**Pullulanase** An enzyme obtained from *Klebsiella pneumoniae*. White crystals. It contains not less than 30 units per mg. One unit is an enzymatic activity to produce 1  $\mu\text{mol}$  of maltotriose from pullulan per minute at pH 5.0 and 30°C.

**Pullulanase TS** A solution of pullulanase containing 10 units per mL.

**Purified hydrochloric acid** See hydrochloric acid, purified.

**Purified methanol** See methanol, purified.

**Purified sulfuric acid** See sulfuric acid, purified.

**Purified water** [Use the water prescribed by the monographs of Purified Water or Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of the relevant test.]

**Pyrazole**  $\text{C}_3\text{H}_4\text{N}_2$  White to pale yellow crystals or crystalline powder.

*Melting point* <2.60>: 67 – 71°C

**Pyridine**  $\text{C}_5\text{H}_5\text{N}$  [K 8777, Special class]

**Pyridine-acetic acid TS** Dilute 20 mL of pyridine with sufficient diluted acetic acid (100) (1 in 25) to make 100 mL. Prepare before use.

**Pyridine, dehydrated**  $\text{C}_5\text{H}_5\text{N}$  To 100 mL of pyridine add 10 g of sodium hydroxide, and allow to stand for 24 hours. Decant the supernatant liquid, and distill.

**Pyridine for Karl Fischer method** See Water Determination <2.48>.

**0.2 mol/L Pyridine-formic acid buffer solution, pH 3.0**

To 15.82 g of pyridine add 900 mL of water, shake well, adjust the pH to 3.0 with diluted formic acid (1 in 2), and add water to make 1000 mL.

**Pyridine-pyrazolone TS** Dissolve, with thorough shaking, 0.1 g of 3-methyl-1-phenyl-5-pyrazolone in 100 mL 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 20 mL of pyridine. Prepare before use.

**Pyridoxine hydrochloride**  $\text{C}_8\text{H}_{11}\text{NO}_3\cdot\text{HCl}$  [Same as the namesake monograph]

**1-(2-Pyridylazo)-2-naphthol**  $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}$  Orange-yellow or orange-red powder.

*Absorbance*—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in methanol to make exactly 100 mL. Pipet 2.0 mL of this solution, and add methanol to make exactly 50 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using methanol as the blank: absorbance at the wavelength of 470 nm is not less than 0.55.

*Melting point* <2.60>: 137 – 140°C

*Purity* Clarity and color of solution—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in 100 mL of methanol: the solution is clear and orange-yellow.

*Residue on ignition* <2.44>: not more than 1.0%.

*Sensitivity*—On adding 50 mL of water, 30 mL of methanol and 10 mL of acetic acid-sodium acetate buffer solution, pH 5.5, to 0.2 mL of a solution of 1-(2-pyridylazo)-2-

naphthol in methanol (1 in 4000), the solution is yellow in color. Add 1 drop of a solution of copper (II) chloride dihydrate (1 in 600) to this solution: the solution is red-purple in color. Add a subsequent 1 drop of diluted 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (1 in 10): the color of the solution changes to yellow again.

**1-(4-Pyridyl)pyridinium chloride hydrochloride**

$C_{10}H_9ClN_2 \cdot HCl$  White to yellowish white, crystalline powder. Very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

*Melting point* <2.60>: 154 – 156°C

**Pyrogallol**  $C_6H_3(OH)_3$  [K 8780, Special class]

**L-Pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride**  $C_{19}H_{26}N_8O_6 \cdot HCl$  White to light powder. Freely soluble in water, in methanol and in acetic acid (100).

*Absorbance* <2.24>  $E_{1\%}^{1\text{cm}}$  (316 nm): 242 – 268 (2 mg, water, 100 mL).

*Optical rotation* <2.49>  $[\alpha]_D^{25}$ : –51 – –56° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

*Purity* Related substances—Dissolve 0.05 g of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu\text{L}$  each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**L-Pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS** Dissolve 25 mg of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride and 0.04 g of D-Mannitol in 2 to 3 mL of water, lyophilize, and add 16.7 mL of water to dissolve. To 1 volume of this solution add 9 volumes of water before use.

**Pyrole**  $C_4H_5N$  Clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

*Specific gravity* <2.56>  $d_{20}^{20}$ : 0.965 – 0.975

**Pyrophosphate buffer solution, pH 9.0** Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol and 40 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 70 mL of water, adjust the pH with a solution of citric acid monohydrate (21 in 100) to exactly 9.0, and add water to make 100 mL.

**0.05 mol/L Pyrophosphate buffer solution, pH 9.0** Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water, adjust the pH with 1 mol/L hydrochloric acid VS to 9.0, and add water to make 50 mL. Adjust the temperature to  $22 \pm 2^\circ\text{C}$  before use.

**Quinapril hydrochloride for assay**  $C_{25}H_{30}N_2O_5 \cdot HCl$  [Same as the monograph Quinapril Hydrochloride. When perform the purity test (2) of Quinapril Hydrochloride, the area of the peaks, having the relative retention time of about

0.5 and about 2.0 to quinapril obtained from the sample solution, is not larger than the peak area of quinapril from the standard solution, the area of peak other than quinapril and above mentioned peak from the sample solution is not larger than 2/5 times the peak area of quinapril from the standard solution, and the total area of the peaks other than quinapril from the sample solution is not larger than 2 times the peak area of quinapril from the standard solution.]

**Quinhydrone**  $C_6H_4(OH)_2 \cdot C_6H_4O_2$  Green crystals or crystalline powder.

*Melting point* <2.60>: 169 – 172°C

**Quinidine sulfate** See quinidine sulfate hydrate.

**Quinidine sulfate hydrate**  $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$  [Same as the namesake monograph]

**Quinine sulfate** See quinine sulfate hydrate.

**Quinine sulfate hydrate**  $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$  [Same as the namesake monograph]

**Quinoline**  $C_9H_7N$  [K 8279, Special class]

**Quinoline TS** Mix 50 mL of quinoline with 300 mL of diluted hydrochloric acid (1 in 6), previously heated, cool, and filter if necessary.

**8-Quinolinol**  $C_9H_7NO$  [K 8775, Special class]

**Raney nickel catalyst** Grayish black powder. An alloy containing 40 to 50% of nickel and 50 to 60% of aluminum.

**Ranitidinediamine**  $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$  White to pale yellow crystalline powder.

*Identification*—Determine the infrared absorption spectrum of ranitidinediamine as directed in the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $2780\text{ cm}^{-1}$ ,  $1637\text{ cm}^{-1}$ ,  $1015\text{ cm}^{-1}$  and  $788\text{ cm}^{-1}$ .

*Content*: not less than 95%. *Assay*—Weigh accurately about 0.1 g of ranitidinediamine, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to green through blue (indicator: crystal violet TS). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 13.62 mg of  $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$

**Rebamipide for assay**  $C_{19}H_{15}ClN_2O_4$  [Same as the monograph Rebamipide. When dried, it contains not less than 99.5% of rebamipide ( $C_{19}H_{15}ClN_2O_4$ ).]

**Reduced iron** See iron powder.

**Reference anti-interleukin-2 antibody for teceleukin**

Monoclonal antibody obtained from a fusion cell strain from mouse spleen cells sensitized to teceleukin and mouse melanoma cells, or alternately, rabbit antiserum towards human interleukin-2, that is purified using affinity chromatography. When determining the neutralizing activity, taking 1 neutralizing unit as the titer that neutralizes one unit of activity of teceleukin, contains at least 2000 neutralizing units per 1 mL.

**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 <2.25>: it exhibits absorption at the wave numbers of about  $3310\text{ cm}^{-1}$ ,  $2130\text{ cm}^{-1}$ ,  $1633\text{ cm}^{-1}$ ,  $1400\text{ cm}^{-1}$ ,  $1261\text{ cm}^{-1}$  and  $711\text{ cm}^{-1}$ .

**Reinecke salt TS** To 20 mL of water add 0.5 g of Reinecke salt monohydrate, shake frequently for 1 hour, then filter. Use within 48 hours.

**Resazurin**  $\text{C}_{12}\text{H}_6\text{NNaO}_4$  Brownish purple powder. It dissolves in water and the solution is purple in color.

**Residue on ignition** <2.44>: not less than 28.5% (1 g).

**Resibufogenin for assay**  $\text{C}_{24}\text{H}_{32}\text{O}_4 \cdot x\text{H}_2\text{O}$  Odorless white crystalline powder.

**Absorbance** <2.24>  $E_{1\text{ cm}}^{1\%}$  (300 nm): 131 – 145 (0.01 g, methanol, 250 mL), dried in a desiccator (silica gel) for 24 hours.

**Purity** Related substances—Weigh accurately 40 mg of resibufogenin for assay and proceed as directed in the Purity under bufalin for assay.

**Content**: not less than 98.0%. **Assay**—Weigh accurately about 10 mg of resibufogenin for assay, previously dried in a desiccator (silica gel) for 24 hours, add methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20  $\mu\text{L}$  of this solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of resibufogenin by the area percentage method.

**Operating conditions**

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

**Column**: A stainless steel column about 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10  $\mu\text{m}$  in particle diameter).

**Column temperature**: A constant temperature of about  $40^\circ\text{C}$ .

**Mobile phase**: A mixture of water and acetonitrile (1:1).

**Selection of column**: Dissolve 0.01 g each of bufalin for assay, cinobufagin for assay and resibufogenin for assay in methanol to make 200 mL. Perform the test with 20  $\mu\text{L}$  of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

**Detection sensitivity**: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of resibufogenin obtained from 20  $\mu\text{L}$  of standard solution (2) can be measured by the automatic integration method and the peak height of resibufogenin from 20  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

**Time span of measurement**: About twice as long as the retention time of resibufogenin beginning after the peak of solvent.

**Resibufogenin for component determination** See resibu-

fogenin for assay.

**Resibufogenin for thin-layer chromatography**

$\text{C}_{24}\text{H}_{32}\text{O}_4 \cdot x\text{H}_2\text{O}$  White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

**Purity** Related substances—Dissolve 5.0 mg of the substance to be tested in exactly 5 mL of acetone. Perform the test with 5  $\mu\text{L}$  of this solution as directed in the Identification under Toad Venom: no other spots than the principal spot of around  $R_f$  0.4 appear.

**Resolving gel for celmoleukin** Prepare the resolving gel in tris buffer solution, pH 8.8 using ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine so the concentrations of acrylamide and sodium lauryl sulfate are 13.5% and 0.1%, respectively.

**Resorcin** See resorcinol.

**Resorcinol**  $\text{C}_6\text{H}_4(\text{OH})_2$  [K 9032, Special class]

**Resorcinol sulfuric acid TS** Dissolve 0.1 g of resorcinol in 10 mL of diluted sulfuric acid (1 in 10).

**Resorcinol TS** Dissolve 0.1 g of resorcinol in 10 mL of hydrochloric acid. Prepare before use.

**Resorcin sulfuric acid TS** See resorcinol sulfuric acid TS.

**Resorcin TS** See resorcinol TS.

**L-Rhamnose monohydrate**  $\text{C}_6\text{H}_{12}\text{O} \cdot \text{H}_2\text{O}$  White crystalline powder having sweet taste. Freely soluble in water, and sparingly soluble in ethanol (95).

**Optical rotation** <2.49>  $[\alpha]_{\text{D}}^{20}$ :  $+7.8 - +8.3^\circ$  (1 g, 20 mL of water, 2 drops of ammonia TS, 100 mm).

**Melting point** <2.60>:  $87 - 91^\circ\text{C}$

**Purity** Related substances—Dissolve 1.0 mg of L-rhamnose monohydrate in 1 mL of water, and add methanol to make exactly 10 mL. Proceed with 20  $\mu\text{L}$  of this solution as directed in the Identification (2) under Acacia: any spot other than the principal spot at the  $R_f$  value of about 0.5 does not appear.

**Rhein for thin-layer chromatography**  $\text{C}_{15}\text{H}_8\text{O}_6$  A yellow powder. Very slightly soluble in acetone, and practically insoluble in water, in methanol, and in ethanol (99.5). Melting point: about  $320^\circ\text{C}$  (with decomposition).

**Identification**—Determine the absorption spectrum of a solution in methanol (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 228 nm and 232 nm, between 255 nm and 259 nm, and between 429 nm and 433 nm.

**Purity** Related substances—Dissolve 1.0 mg in 10 mL of acetone, and perform the test with 2  $\mu\text{L}$  of this solution as directed in the Identification (1) under Daiokanzoto Extract: no spot other than the principal spot ( $R_f$  value is about 0.3) appears.

**Rhyncophylline for assay**  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$  Rhyncophylline for thin-layer chromatography. It meets the following requirements.

**Absorbance** <2.24>:  $E_{1\text{ cm}}^{1\%}$  (245 nm): 473 – 502 (5 mg of the dried substance in a desiccator (silica gel) for 24 hours, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

**Purity** Related substances—Dissolve 5 mg of rhyncophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample

solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid-chromatography <2.01> according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than rhyncophylline obtained from the sample solution is not larger than the peak area of rhyncophylline from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Uncaria Hook.

Time span of measurement: About 4 times as long as the retention time of rhyncophylline, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhyncophylline obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Uncaria Hook.

**Rhyncophylline for component determination** See rhyncophylline for assay.

**Rhyncophylline for thin-layer chromatography**

$C_{22}H_{28}N_2O_4$  White crystals or crystalline powder. Slightly soluble in ethanol (99.5) and in acetone, and practically insoluble in water. Melting point: 205 – 209°C.

*Identification*—Determine the absorption spectrum of a solution of rhyncophylline for thin-layer chromatography in a mixture of methanol and dilute acetic acid (7:3) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 242 nm and 246 nm.

*Purity* Related substances—Dissolve 1.0 mg of rhyncophylline for thin-layer chromatography in 1 mL of acetone, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): no spot other than the principal spot at around *Rf* 0.5 appears.

**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]

**Risperidone for assay**  $C_{23}H_{27}FN_4O_2$  [Same as the monograph Risperidone. It contains not less than 99.5% of risperidone ( $C_{23}H_{27}FN_4O_2$ ), calculated on the dried basis.]

**Ritodrine hydrochloride**  $C_{17}H_{21}NO_3 \cdot HCl$  [Same as the namesake monograph]

**Rose Bengal** See Microbial Limit Test for Crude Drugs <5.02>.

**Rose Bengal TS** See Microbial Limit Test for Crude Drugs <5.02>.

**Rosmarinic acid for assay** Rosmarinic acid for thin-layer chromatography. However, it meets the following requirements:

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (325 nm): 502 – 534 (5 mg, water, 500 mL).

*Purity* Related substances—Conduct this procedure using light-resistant vessels. Dissolve 5 mg of rosmarinic acid for assay in 20 mL of the mobile phase, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than rosmarinic acid from the sample solution is not larger than the peak area of rosmarinic acid from the standard solution. Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 240 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  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: Adjust the flow rate so that the retention time of rosmarinic acid is about 14 minutes.

Time span of measurement: About 4 times as long as the retention time of rosmarinic acid.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of rosmarinic acid obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rosmarinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rosmarinic acid is not more than 1.5%.

**Rosmarinic acid for component determination** See rosmarinic acid for assay.

**Rosmarinic acid for thin-layer chromatography**

$C_{18}H_{16}O_8$  White to pale yellow crystals or crystalline powder. Freely soluble in ethanol (99.5), and slightly soluble in water. Melting point: about 170°C (with decomposition).

*Identification*—Determine the absorption spectrum of a solution of rosmarinic acid for thin-layer chromatography (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm and between 322 nm and 326 nm.

**Purity** Related substances—Conduct this procedure using light-resistance vessels. Dissolve 10 mg of rosmarinic acid for thin-layer chromatography in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Proceed with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Hangekobokuto Extract: the spot other than the principal spot of around *R<sub>f</sub>* 0.5 from the sample solution is not more intense than the spot from the standard solution.

**Roxatidine acetate hydrochloride**  $C_{19}H_{28}N_2O_4 \cdot HCl$   
[Same as the namesake monograph]

**RPMI-1640 powdered medium** Powder medium for cell culture containing 6 g of sodium chloride, 400 mg of potassium chloride, 800 mg of sodium dihydrogen phosphate, 100 mg of anhydrous calcium nitrate, 49 mg of anhydrous magnesium sulfate, 2 g of dextrose, 200 mg of L-arginine, 1 mg of glutathione, 50 mg of L-isoleucine, 15 mg of L-phenylalanine, 5 mg of L-tryptophan, 0.2 mg of biotin, 1 mg of nicotinamide, 1 mg thiamine hydrochloride, 300 mg of L-glutamine, 56.8 mg of L-asparagine, 10 mg of glycine, 50 mg of L-leucine, 20 mg of L-proline, 20 mg of L-tyrosine, 0.25 mg of D-calcium pantothenate, 5  $\mu$ g of cyanocobalamin, 1 mg of aminobenzoic acid, 20 mg of L-aspartic acid, 15 mg of L-histidine, 40 mg of L-lysine hydrochloride, 30 mg of L-serine, 20 mg of L-valine, 1 mg of folic acid, 1 mg of pyridoxine hydrochloride, 20 mg of L-glutamic acid, 20 mg of L-hydroxyproline, 15 mg of L-methionine, 20 mg of L-threonine, 3 mg of choline chloride, 35 mg of *i*-inositol, 0.2 mg of riboflavin, 59 mg of L-cystine, and 5 mg of phenol red.

**Saccharated pepsin** [Same as the namesake monograph]

**Saikosaponin a for assay** Use saikosaponin a for thin-layer chromatography meeting the following additional specifications.

**Purity** Related substances—

(1) Dissolve 2.0 mg of saikosaponin a for assay in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed in the Purity (2) under Bupleurum Root: the spot other than the principal spot around *R<sub>f</sub>* 0.4 is not larger and not more intense than the spot obtained with the standard solution.

(2) Dissolve 10 mg of saikosaponin a for assay in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin a is not more than the peak area of saikosaponin a obtained with the standard solution.

**Operating conditions**

Detector, and column: Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

Column temperature: A constant temperature of about

40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin a is about 16 minutes.

Time span of measurement: About 6 times as long as the retention time of saikosaponin a beginning after the solvent peak.

**System suitability**

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin a obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin a for assay and saikosaponin b<sub>2</sub> for assay in methanol to make 100 mL. When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, saikosaponin a and saikosaponin b<sub>2</sub> 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 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin a is not more than 1.0%.

**Saikosaponin a for component determination** See saikosaponin a for assay.

**Saikosaponin a for thin-layer chromatography** A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 225 – 232°C (with decomposition).

**Absorbance** <2.24>  $E_{1\text{cm}}^{1\%}$  (206 nm): 60–68 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

**Purity** Related substances—Dissolve 1.0 mg of saikosaponin a for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification (2) under Bupleurum Root: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Saikosaponin b<sub>2</sub> for assay**  $C_{42}H_{68}O_{13}$  Saikosaponin b<sub>2</sub> for thin-layer chromatography. It meets the following requirements.

**Purity** Related substances—Dissolve 5 mg in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of saikosaponin b<sub>2</sub> and solvent obtained from the sample solution is not larger than the peak area of saikosaponin b<sub>2</sub> from the standard solution.

**Operating conditions**

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Saireito Extract.

Time span of measurement: About 6 times as long as the retention time of saikosaponin b<sub>2</sub>.

**System suitability**

Test for required detectability: To exactly 1 mL of the

standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of saikosaponin b<sub>2</sub> obtained from 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that from 10  $\mu$ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Saireito Extract.

**Saikosaponin b<sub>2</sub> for component determination** See saikosaponin b<sub>2</sub> for assay.

**Saikosaponin b<sub>2</sub> for thin-layer chromatography**

C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> White crystals or crystalline powder. Freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water. Melting point: about 240°C

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (252 nm): 352 – 424 [5 mg dried in a desiccator (in vacuum, silica gel) for 24 hours, methanol, 250 mL].

*Purity* Related substances—Dissolve 2 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Proceed the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (1) under Saireito Extract: the spot other than the principle spot, having *R<sub>f</sub>* value of about 0.3, obtained from the sample solution is not more intense than the spot from the standard solution.

**Saikosaponin d for assay** C<sub>42</sub>H<sub>68</sub>O<sub>13</sub> A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 240°C.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (206 nm): 63 – 71 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

*Purity* Related substances—

(1) Dissolve 2.0 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with 10  $\mu$ L each of the sample solution and standard solution as directed in the Identification (2) under Bupleurum Root: the spot other than the principal spot around *R<sub>f</sub>* 0.4 is not larger and not more intense than the spot obtained with the standard solution.

(2) Dissolve 10 mg in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin d and the solvent is not more than the peak area of saikosaponin d obtained with the standard solution.

Operating conditions

Detector, and column: Proceed as directed in the operating conditions in the Assay under Bupleurum Root.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin d is about 13 minutes.

Time span of measurement: About 4 times as long as the retention time of saikosaponin d beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin d obtained with 20  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 20  $\mu$ L of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin d for assay and saikosaponin a for assay in methanol to make 100 mL. When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, saikosaponin a and saikosaponin d 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 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin d is not more than 1.0%.

**Saikosaponin d for component determination** See saikosaponin d for assay.

**Salicylaldazine** C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> Dissolve 0.30 g of hydrazinium sulfate in 5 mL of water. To this solution add 1 mL of acetic acid (100) and 2 mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well, and allow to stand until a yellow precipitate is produced. Extract with two 15 mL portions 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 (3:2), 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* <2.60>: 213 – 219°C

*Purity* Related substances—Dissolve 0.09 g of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and perform the test with this solution as directed in the Purity (6) under Povidone: any spot other than the principal spot does not appear.

**Salicylaldehyde** HOC<sub>6</sub>H<sub>4</sub>CHO [K 8390, Special class]

**Salicylamide** C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub> White crystals or crystalline powder, and it is odorless and tasteless. Very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), soluble in propylene glycol, sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform. It dissolves in sodium hydroxide TS.

*Melting point* <2.60>: 139 – 143°C

*Purity* Ammonium <1.02>—Shake 1.0 g of salicylamide with 40 mL of water, and filter through filter paper previously washed well with water. Discard the first 10 mL of the filtrate, transfer the subsequent 20 mL to a Nessler tube, and add water to make 30 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: transfer 2.5 mL of Standard Ammonium Solution to a Nessler tube, and add water to make 30 mL.

*Loss on drying* <2.41>: not more than 0.5% (1 g, silica gel, 4 hours).

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Content*: not less than 98.5%. Assay—Weigh accurately

about 0.2 g of salicylamide, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution of 70 mL of *N,N*-dimethylformamide in 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.71 mg of  $C_7H_7NO_2$

**Salicylic acid**  $HOC_6H_4COOH$  [K 8392, Special class]

**Salicylic acid for assay**  $HOC_6H_4COOH$  [K 8392, Special class]

**Salicylic acid TS** Dissolve 0.1 g of salicylic acid in 10 mL of sulfuric acid. Prepare before use.

**Santonin**  $C_{15}H_{18}O_3$  [Same as the namesake monograph]

**Santonin for assay** [Same as the monograph Santonin. It contains not less than 99.0% of santonin ( $C_{15}H_{18}O_3$ ).]

**Sarpogrelate hydrochloride**  $C_{24}H_{31}NO_6.HCl$  [Same as the namesake monograph]

**Schisandrin for thin-layer chromatography**  $C_{24}H_{32}O_7$   
White crystals for crystalline powder. Freely soluble in methanol and diethyl ether, and practically insoluble in water.

*Melting point* <2.60>: 130 – 135°C

*Purity* Related substances—Dissolve 1.0 mg of schisandrin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 5  $\mu$ L of this solution as directed in the Identification under Schisandra Fruit: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.4 does not appear.

**Scopolamine hydrobromide** See scopolamine hydrobromide hydrate.

**Scopolamine hydrobromide for thin-layer chromatography** See scopolamine hydrobromide hydrate for thin-layer chromatography.

**Scopolamine hydrobromide hydrate**  
 $C_{17}H_{21}NO_4.HBr.3H_2O$  [Same as the namesake monograph]

**Scopolamine hydrobromide hydrate for thin-layer chromatography**  $C_{17}H_{21}NO_4.HBr.3H_2O$  [Same as the monograph Scopolamine Hydrobromide Hydrate. Proceed as directed in the Identification (3) under Opium Alkaloids and Atropine Injection: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.7 does not appear.

**Sea sand** A mixture of white, grey, brown or black grains, 0.3 to 1.0 mm in particle size.

**2nd Fluid for disintegration test** To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 118 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. It is clear and colorless, and has a pH about 6.8.

**2nd Fluid for dissolution test** A mixture of phosphate buffer solution, pH 6.8 and water (1:1).

**Selenious acid**  $H_2SeO_3$  Colorless or white crystals. It is hygroscopic.

*Identification*—(1) Dissolve 0.2 g of selenious acid in 20 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL of tin (II) chloride TS: a red

precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

Preserve in a light-resistant tight container.

**Selenious acid-sulfuric acid TS** Dissolve 50 mg of selenious acid in 10 mL of sulfuric acid.

**Selenium** Se [K 8598, Special class]

**Selenium dioxide**  $SeO_2$  White crystals or crystalline powder.

*Identification*—(1) To 10 mL of a solution of selenium dioxide (1 in 100) add 2 mL of tin (II) chloride TS: a red precipitate is produced.

(2) To 10 mL of a solution of selenium dioxide (1 in 100) add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of potassium iodide TS: a brown color is produced.

*Content*: not less than 97.0%. *Assay*—Weigh accurately about 0.6 g of selenium dioxide, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution into an iodine bottle, add 80 mL of water, 3 g of potassium iodide and 5 mL of diluted hydrochloric acid (2 in 3), allow to stand at a dark place for 5 minutes, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS = 2.774 mg of  $SeO_2$

**Semicarbazide acetate TS** Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30 mL of methanol in a flask, heat on a water bath for 2 hours, cool to 20°C, and filter. To the filtrate add methanol to make 100 mL. Preserve in a cold place. Do not use the solution showing a yellow color.

**Semicarbazide hydrochloride**  $H_2NNHCONH_2.HCl$   
White to light yellow crystals.

*Identification* (1) To 10 mL of a solution of semicarbazide hydrochloride (1 in 100) add 1 mL of silver nitrate TS: white precipitates appear.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3420  $cm^{-1}$ , 3260  $cm^{-1}$ , 2670  $cm^{-1}$ , 1684  $cm^{-1}$ , 1582  $cm^{-1}$ , 1474  $cm^{-1}$ , 1386  $cm^{-1}$ , 1210  $cm^{-1}$ , 1181  $cm^{-1}$ , 770  $cm^{-1}$  and 719  $cm^{-1}$ .

**Sennoside A for thin-layer chromatography**  $C_{42}H_{38}O_{20}$   
Yellow crystalline powder. Insoluble in water, in chloroform and in diethyl ether, and practically insoluble in methanol and in acetone. Melting point: 200 – 240°C (with decomposition).

*Purity* Related substances—Dissolve 1.0 mg of sennoside A for thin-layer chromatography in exactly 4 mL of a mixture of tetrahydrofuran and water (7:3), and perform the test with 80  $\mu$ L of this solution as directed in the identification under Rhubarb: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.3 does not appear.

**L-Serine**  $C_3H_7NO_3$  [K 9105, Special class]

**Sesame oil** [Same as the namesake monograph]

**Sesamin for thin-layer chromatography**  $C_{20}H_{18}O_6$

White crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

*Melting point* <2.60>: 122 – 124°C

*Identification* Determine the absorption spectrum of a solution of Sesame in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm and between 285 nm and 289 nm.

*Purity* Related substances—Dissolve 2.0 mg in 2 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this as the standard solution. Perform the test with 5  $\mu$ L each of the sample solution and standard solution as directed in the Identification under Sesame: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**[6]-Shogaol for assay**  $C_{17}H_{24}O_3$  [6]-Shogaol for thin-layer chromatography. It meets the following requirements.

*Absorbance* <2.24>:  $E_{1\text{cm}}^{1\%}$  (225 nm): 727 – 781 (5 mg, ethanol (99.5), 500 mL).

*Purity* Related substances—Dissolve 5 mg of [6]-shogaol for assay in 10 mL of a mixture of acetonitrile and water (2:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (2:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area of each solution by the automatic integration method: the total area of the peaks other than [6]-shogaol obtained from the sample solution is not larger than the peak area of [6]-shogaol from the standard solution.

*Operating conditions*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions under Assay (2) of Mukoi-Daikenchuto Extract.

Time span of measurement: 3 times as long as the retention time of [6]-shogaol, beginning after the solvent peak.

*System suitability*

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of acetonitrile and water (2:1) to make exactly 20 mL. Confirm that the peak area of [6]-shogaol obtained with 10  $\mu$ L of this solution is equivalent to 3.5 to 6.5% of that with 10  $\mu$ L of the standard solution.

*System performance*: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5%, respectively.

*System repeatability*: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

**[6]-Shogaol for thin-layer chromatography**  $C_{17}H_{24}O_3$  A pale yellow oil. Miscible with methanol, ethanol (99.5) and with diethyl ether, and practically insoluble in water.

*Purity* Related substances—Dissolve 1.0 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L on a plate of

silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: no spot other than the principal spot at around *Rf* 0.5 appears.

**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.

*Loss on ignition* <2.43>: not more than 6% (2 g, 950  $\pm$  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.

**Siliceous earth** [K 8330, Diatomaceous earth, First class]

**Silicone oil** Colorless clear liquid, having no odor.

*Viscosity* <2.53>: 50 – 100 mm<sup>2</sup>/s.

**Silicone resin** 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 150 mL of carbon tetrachloride for 3 hours. The kinematic viscosity of the residual liquid, obtained by evaporating carbon tetrachloride from the extract on a water bath, is 100 to 1100 mm<sup>2</sup>/s (25°C). Its refractive index is 1.400 to 1.410 (25°C).

*Specific gravity* <2.56> *d*: 0.98 – 1.02

*Loss on drying* <2.41>: 0.45 – 2.25 g with the extracted residue obtained in the Viscosity and refractive index (100°C, 1 hour).

**Silicotungstic acid 26-water**  $SiO_2 \cdot 12WO_3 \cdot 26H_2O$

White to slightly yellowish, crystals. Deliquescent. Very soluble in water and in ethanol (95).

*Purity* Clarity and color of solution—a solution (1 in 20) is clear and colorless.

*Loss on ignition* <2.43>: 14 – 15% (2 g, dry at 110°C for 2 hours then 700 – 750°C, constant mass).

**Silver chromate-saturated potassium chromate TS** Dissolve 5 g of potassium chromate in 50 mL of water, add silver nitrate TS until a pale red precipitate is produced, and filter. To the filtrate add water to make 100 mL.

**Silver diethyldithiocarbamate** See silver *N,N*-diethyldithiocarbamate.

**Silver nitrate**  $AgNO_3$  [K 8550, Special class]

**Silver nitrate-ammonia TS** Dissolve 1 g of silver nitrate in 20 mL 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 1000 mL (0.1 mol/L). Preserve in light-resistant containers.

**Silver *N,N*-diethyldithiocarbamate**  $C_5H_{10}AgNS_2$

[K 9512]

**Soda lime** [K 8603, First class]**Sodium Na** [K 8687, 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 100 mL of water, and add 68 mL of 0.1 mol/L hydrochloric acid VS, 150 mL of acetone and water to make 500 mL.**Sodium acetate, anhydrous**  $\text{CH}_3\text{COONa}$  [K 8372, Special class]**Sodium acetate trihydrate**  $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$  [K 8371, Special class]**Sodium acetate TS** Dissolve 13.6 g of sodium acetate trihydrate in water to make 100 mL (1 mol/L).**Sodium azide**  $\text{NaN}_3$  [K 9501, Special class]**Sodium benzoate** [Same as the namesake monograph]**Sodium bicarbonate** See sodium hydrogen carbonate.**Sodium bicarbonate for pH determination** See sodium hydrogen carbonate for pH determination.**Sodium bicarbonate TS** See sodium hydrogen carbonate TS.**7% Sodium bicarbonate injection** [Same as the monograph Sodium Bicarbonate Injection. However, labeled amount should be 7 w/v%.]**Sodium bismuthate** See bismuth sodium trioxide.**Sodium bisulfite** See sodium hydrogen sulfite.**Sodium bisulfite TS** See sodium hydrogen sulfite TS.**Sodium bitartrate** See sodium hydrogen tartrate monohydrate.**Sodium bitartrate TS** See sodium hydrogen tartrate TS.**Sodium borate** See sodium tetraborate decahydrate.**Sodium borate for pH determination** See sodium tetraborate decahydrate for pH determination.**Sodium borohydride**  $\text{NaBH}_4$  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 20 mL of diluted sodium hydroxide TS (3 in 10), and add water to make exactly 500 mL. Pipet 20 mL of this solution, put in a glassstopped iodine flask, and cool in ice. Add exactly 40 mL of iodine TS, allow to stand at a dark place for 10 minutes, add exactly 10 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 0.4729 mg of  $\text{NaBH}_4$

**Sodium bromide**  $\text{NaBr}$  [K 8514, Special class]**Sodium carbonate** See sodium carbonate decahydrate.**Sodium carbonate, anhydrous**  $\text{Na}_2\text{CO}_3$  [K 8625, Sodium carbonate, Special class]**Sodium carbonate decahydrate**  $\text{Na}_2\text{CO}_3\cdot 10\text{H}_2\text{O}$  [K 8624, Special class]**Sodium carbonate for pH determination**  $\text{Na}_2\text{CO}_3$  [K 8625, for pH determination]**Sodium carbonate (standard reagent)**  $\text{Na}_2\text{CO}_3$  [K 8005, Standard reagent for volumetric analysis]**Sodium carbonate TS** Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).**0.55 mol/L Sodium carbonate TS** Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.**Sodium chloride**  $\text{NaCl}$  [K 8150, Special class]**Sodium chloride (standard reagent)**  $\text{NaCl}$  [K 8005, Standard reagent for volumetric analysis]**Sodium chloride TS** Dissolve 10 g of sodium chloride in water to make 100 mL.**0.1 mol/L Sodium chloride TS** Dissolve 6 g of sodium chloride in water to make 1000 mL.**0.2 mol/L Sodium chloride TS** Dissolve 11.7 g of sodium chloride in water to make 1000 mL.**1 mol/L Sodium chloride TS** Dissolve 29.22 g of sodium chloride in water to make 500 mL.**Sodium citrate** See sodium citrate hydrate.**Sodium citrate hydrate**  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot 2\text{H}_2\text{O}$  [K 8288, Trisodium citrate dihydrate, or same as the namesake monograph]**Sodium cobaltinitrite** See sodium hexanitrocobaltate (III).**Sodium cobaltinitrite TS** See sodium hexanitrocobaltate (III) TS.**Sodium 1-decanesulfonate**  $\text{C}_{10}\text{H}_{21}\text{NaO}_3\text{S}$  A white powder.

*Purity* Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless.

*Loss on drying* <2.41>: 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 50 mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion-exchange resin (0.3 to 1.0 mm, H type) at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 24.43 mg of  $\text{C}_{10}\text{H}_{21}\text{NaO}_3\text{S}$

**0.0375 mol/L Sodium 1-decanesulfonate TS** Dissolve 3.665 g of sodium 1-decanesulfonate in 400 mL of water.**Sodium desoxycholate**  $\text{C}_{24}\text{H}_{39}\text{NaO}_4$  White, odorless,

crystalline powder.

**Identification**—Determine the infrared absorption spectrum of sodium desoxycholate, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $3400\text{ cm}^{-1}$ ,  $2940\text{ cm}^{-1}$ ,  $1562\text{ cm}^{-1}$  and  $1408\text{ cm}^{-1}$ .

**Purity** Related substances—Dissolve 0.10 g of sodium desoxycholate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu\text{L}$  each of the sample solution and 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  $105^\circ\text{C}$  for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Sodium 2,6-dichloroindophenol-sodium acetate TS** Mix before use an equal volume of sodium 2,6-dichloroindophenol dehydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0.

**Sodium diethyldithiocarbamate** See sodium *N,N*-diethyldithiocarbamate trihydrate.

**Sodium *N,N*-diethyldithiocarbamate trihydrate** ( $\text{C}_2\text{H}_5$ )<sub>2</sub>NCS<sub>2</sub>Na.3H<sub>2</sub>O [K 8454, Special class]

**Sodium di-2-ethylhexyl sulfosuccinate** C<sub>8</sub>H<sub>17</sub>COOCH<sub>2</sub>(C<sub>8</sub>H<sub>17</sub>COO)CHSO<sub>3</sub>Na White or translucent white mucilaginous soft masses. Sparingly soluble in water.

**Purity** Clarity and color of solution—A solution prepared by dissolving 1.0 g in 100 mL of water is clear and colorless.

**Loss on drying** <2.41>: not more than 5.0% (1 g,  $105^\circ\text{C}$ , 2 hours).

**Sodium dihydrogen phosphate** See sodium dihydrogen phosphate dihydrate.

**Sodium dihydrogen phosphate anhydrous** NaH<sub>2</sub>PO<sub>4</sub> A white, powder or crystalline powder. Freely soluble in water, and very slightly soluble in ethanol (99.5). It has a hygroscopic property.

A solution is acidic.

**Sodium dihydrogen phosphate dihydrate** NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O [K 9009, Special class]

**Sodium dihydrogen phosphate TS, pH 2.2** Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 800 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL.

**Sodium dihydrogen phosphate TS, pH 2.5** Dissolve 2.7 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

**0.05 mol/L Sodium dihydrogen phosphate TS, pH 2.6** Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to exactly 2.6 and add water to make 1000 mL.

**0.05 mol/L Sodium dihydrogen phosphate TS, pH 3.0**

Dissolve 3.45 g of sodium dihydrogen phosphate dihydrate in 500 mL of water (solution A). Dilute 2.45 g of phosphoric acid with water to make 500 mL (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 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

**0.05 mol/L Sodium dihydrogen phosphate TS** Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

**0.1 mol/L Sodium dihydrogen phosphate TS** Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, adjust to a pH of 5.8 exactly with sodium hydroxide TS, and add water to make 500 mL.

**2 mol/L Sodium dihydrogen phosphate TS** Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

**Sodium disulfite** Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> [K 8501, First class]

**Sodium disulfite TS** Dissolve 0.10 g of sodium disulfite in 10 mL of 1 mol/L hydrochloric acid TS, and add acetone to make 100 mL.

**Sodium dithionite** Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> A white to grayish white crystalline powder, having a strong irritating odor. It is decomposed with moisture or atmospheric oxygen.

**Identification**—(1) Dissolve 0.5 g of sodium dithionite in 50 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 1 mL of copper (II) sulfate TS: a grayish brown color 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 dodecylbenzene sulfonate** C<sub>18</sub>H<sub>29</sub>SO<sub>3</sub>Na White, crystalline powder or mass.

**pH** <2.54>—The pH of a solution of 0.5 g of sodium dodecylbenzene sulfonate in 50 mL of freshly boiled and cooled water is between 5.0 and 7.0. Measure the pH at  $25^\circ\text{C}$  passing nitrogen with stirring.

**Loss on drying** <2.41>: not more than 0.5% (1 g,  $105^\circ\text{C}$ , 2 hours).

**Content**: not less than 99.0%. **Assay**—Weigh accurately about 40 mg of sodium dodecylbenzene sulfonate, previously dried, and perform the test as directed in (4) Sulfur in the Procedure of determination under Oxygen Flask Combustion Method <1.06>, using a mixture of 20 mL of water and 2 mL of strong hydrogen peroxide water as absorbing solution.

Each mL of 0.01 mol/L barium perchlorate VS  
= 1.742 mg of C<sub>18</sub>H<sub>29</sub>SO<sub>3</sub>Na

**Sodium fluoride** NaF [K 8821, Special class]

**Sodium fluoride (standard reagent)** NaF [K 8005, Standard reagent for volumetric analysis]

**Sodium fluoride TS** Dissolve 0.5 g of sodium fluoride in 100 mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.

**Sodium glycocholate for thin-layer chromatography**

$C_{26}H_{42}NNaO_6 \cdot xH_2O$  White to pale brown crystalline powder or powder. Freely soluble in water and in methanol, and slightly soluble in ethanol (99.5). Melting point: about 260°C (with decomposition).

**Identification**—Determine the infrared absorption spectrum of sodium glycocholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, it exhibits absorption at the wave numbers of about 2940  $cm^{-1}$ , 1640  $cm^{-1}$ , 1545  $cm^{-1}$ , 1450  $cm^{-1}$ , 1210  $cm^{-1}$ , 1050  $cm^{-1}$ , and 600  $cm^{-1}$ .

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ : +25 – +35° (60 mg, methanol, 20 mL, 100 mm).

**Purity** Related substances—Dissolve 5 mg of sodium glycocholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Proceed with 5  $\mu L$  each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot at the *R<sub>f</sub>* value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

**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 10 mL of water: the solution is clear and colorless.

**Loss on drying** <2.41>: 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 50 mL of water, transfer to a chromatographic column, prepared by packing a chromatographic tube 9 mm in inside diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600  $\mu m$  in particle diameter, H type), and flow at a flow rate of about 4 mL per minute. Wash the column at the same flow rate with 150 mL of water, combine the washings with the effluent solution, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 20.23 mg of  $C_7H_{15}NaO_3S$

**Sodium 1-hexane sulfonate**  $C_6H_{13}NaO_3S$  White, crystals or crystalline powder.

**Loss on drying** <2.41>: not more than 3.0% (1 g, 105°C, 2 hours).

**Content**: not less than 98.0%. **Assay**—Weigh accurately about 0.4 g of sodium 1-hexane sulfonate, previously dried, and dissolve in 25 mL of water. Transfer 15–20 mL of this solution into a chromatographic column about 11 mm in diameter and about 500 mm in height of strongly acidic ion exchange resin for column chromatography (246  $\mu m$  to 833  $\mu m$  in particle diameter, type H), and elute at the rate of about 5 – 10 mL per minute, then wash the column with five 50-mL portions of water at the rate of about 5 – 10 mL per minute. Combine the washings to the eluate, and titrate <2.50> with

0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 18.82 mg of  $C_6H_{13}NaO_3S$

**Sodium hexanitrocobaltate (III)**  $Na_3Co(NO_2)_6$   
[K 8347, Special class]

**Sodium hexanitrocobaltate (III) TS** Dissolve 10 g of sodium hexanitrocobaltate (III) in water to make 50 mL, and filter if necessary. Prepare before use.

**Sodium hydrogen carbonate**  $NaHCO_3$  [K 8622, Special class]

**Sodium hydrogen carbonate for pH determination**  $NaHCO_3$  [K 8622, Sodium hydrogen carbonate, for pH determination]

**Sodium hydrogen carbonate TS** Dissolve 5.0 g of sodium hydrogen carbonate in water to make 100 mL.

**7% Sodium hydrogen carbonate injection** See 7% sodium bicarbonate injection.

**Sodium hydrogen sulfite** [K 8059, Special class]

**Sodium hydrogen sulfite TS** Dissolve 10 g of sodium hydrogen sulfite in water to make 30 mL. Prepare before use.

**Sodium hydrogen tartrate monohydrate**  $NaHC_4H_4O_6 \cdot H_2O$  [K 8538, Sodium hydrogentartrate monohydrate, Special class]

**Sodium hydrogen tartrate TS** Dissolve 1 g of sodium bitartrate in water to make 10 mL (0.5 mol/L). Prepare before use.

**Sodium hydroxide**  $NaOH$  [K 8576, 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 100 mL.

**Sodium hydroxide-methanol TS** Dissolve by thorough shaking 4 g of sodium hydroxide in methanol to make 100 mL. To the supernatant liquid obtained by centrifugation add methanol to make 500 mL. Prepare before use.

**Sodium hydroxide TS** Dissolve 4.3 g of sodium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

**Sodium hydroxide TS, dilute** Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use (0.1 mol/L).

**0.01 mol/L Sodium hydroxide TS** Dilute 10 mL of sodium hydroxide TS with water to make 1000 mL. Prepare before use.

**0.05 mol/L Sodium hydroxide TS** To 10 mL of 0.5 mol/L sodium hydroxide TS add water to make 100 mL.

**0.2 mol/L Sodium hydroxide TS** Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use.

**0.5 mol/L Sodium hydroxide TS** Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**2 mol/L Sodium hydroxide TS** Dissolve 86 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**4 mol/L Sodium hydroxide TS** Dissolve 168 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**6 mol/L Sodium hydroxide TS** Dissolve 252 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

**8 mol/L Sodium hydroxide TS** Dissolve 336 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

**Sodium hypobromite TS** To 8 mL of bromine TS add 25 mL of water and 25 mL of sodium carbonate TS. Prepare before use.

**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 1000 mL. 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** 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 10 mL of sodium hypochlorite TS for ammonium limit test, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution to a glass-stoppered flask, add 90 mL of water, then add 2 g of potassium iodide and 6 mL of diluted acetic acid (1 in 2), stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS  
= 3.722 mg of NaClO.

**Sodium lauryl sulfate** [Same as the namesake monograph]

**Sodium lauryl sulfate TS** Dissolve 100 g of sodium lauryl sulfate in 900 mL of water, add 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.2% Sodium lauryl sulfate TS** Dissolve 0.1 g of sodium lauryl sulfate in 0.1 mol/L of pH 7.0 sodium phosphate buffer to make 50 mL.

**Sodium metabisulfite** See sodium disulfite.

**Sodium metabisulfite TS** See sodium disulfite TS.

**Sodium, metallic** Na [K 8687, Sodium, Special class]

**Sodium 1-methyl-1H-tetrazole-5-thiolate** See sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate.

**Sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate**

$C_2H_3N_4NaS \cdot 2H_2O$  White, crystals or crystalline powder.

*Melting point* <2.60>: 90 – 94°C

*Purity* Related substances—Dissolve 10 mg of sodium 1-methyl-1H-tetrazole-5-thiolate dihydrate in 10 mL of water, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

**Sodium molybdate** See sodium molybdate (VI) dihydrate.

**Sodium molybdate (VI) dihydrate**  $Na_2MoO_4 \cdot 2H_2O$   
[K 8906, 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  $\beta$ -naphthoquinone sulfonate in methanol to make 100 mL.

**Sodium  $\beta$ -naphthoquinone 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* <2.41>: Not more than 2.0% (1 g, in vacuum, 50°C).

*Residue on ignition* <2.44>: 26.5 – 28.0% (1 g, after drying).

**Sodium nitrate**  $NaNO_3$  [K 8562, Special class]

**Sodium nitrite**  $NaNO_2$  [K 8019, Special class]

**Sodium nitrite TS** Dissolve 10 g of sodium nitrite in water to make 100 mL. Prepare before use.

**Sodium nitroprusside** See sodium pentacyanonitrosylferate (III) dihydrate.

**Sodium nitroprusside TS** See sodium pentacyanonitrosylferate (III) TS.

**Sodium 1-nonanesulfonate**  $CH_3(CH_2)_8SO_3Na$  White crystalline powder. Freely soluble in water.

*Loss on drying* <2.41>: Not more than 1.0% (1 g, 105°C, 3 hours).

*Residue on ignition* <2.44>: 30 – 32% (0.5 g).

**Sodium 1-octane sulfonate**  $CH_3(CH_2)_7SO_3Na$  White crystals or powder.

*Residue on ignition* <2.44>: 32.2 – 33.0% (1.0 g).

**Sodium oxalate (standard reagent)**  $C_2O_4Na_2$  [K 8005, Standard reagent for volumetric analysis]

**Sodium pentacyanoammine ferroate (II) *n*-hydrate**  $Na_3[Fe(CN)_5NH_3] \cdot nH_2O$  A light yellow to light green-yellow crystalline powder.

*Identification*—(1) Dissolve 0.2 g of sodium pentacyanoammine ferroate (II) *n*-hydrate in 5 mL of water, add 2 mL of sodium hydroxide solution (1 in 10), and heat: ammonia gas is evolved and a brown precipitate is produced.

(2) Dissolve 0.25 g of sodium pentacyanoammine ferro-

ate (II) *n*-hydrate in 20 mL of water. To 1 mL of this solution add 0.2 mL of iron (II) sulfate TS: a green-blue color develops, which changes to a dark blue color on the addition of 2 drops of diluted sodium hypochlorite TS (2 in 5) and 0.2 mL of acetic acid (100).

**Sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS** Mix an equal volume of a solution of sodium pentacyanonitrosylferrate (III) dihydrate (1 in 10), a solution of potassium hexacyanoferrate (III) (1 in 10) and a solution of sodium hydroxide (1 in 10), and allow to stand for 30 minutes. Use after the color of the solution is changed from a dark red to yellow. Prepare before use.

**Sodium pentacyanonitrosylferrate (III) dihydrate**  
 $\text{Na}_2\text{Fe}(\text{CN})_5(\text{NO}) \cdot 2\text{H}_2\text{O}$  [K 8722, Special class]

**Sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS, dilute** To 5 mL of a solution of pentacyanonitrosylferrate (III) dihydrate (3 in 50) add 5 mL of a solution of potassium hexacyanoferrate (III) (13 in 200) and 2.5 mL of a solution of sodium hydroxide (1 in 10), add water to make 25 mL, mix, and use after changing the color of the solution from a dark red to yellow. Prepare before use.

**Sodium pentacyanonitrosylferrate (III) TS** Dissolve 1 g of sodium pentacyanonitrosylferrate (III) dihydrate in water to make 20 mL. Prepare before use.

**Sodium 1-pentane sulfonate**  $\text{C}_5\text{H}_{11}\text{NaO}_3\text{S}$  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 10 mL of water: the solution is colorless and clear.

**Water** <2.48>: not more than 3.0% (0.2 g).

**Content:** not less than 99.0%, calculated on the anhydrous basis. **Assay**—Dissolve about 0.3 g of sodium 1-pentane sulfonate, accurately weighed, in 50 mL of water. Transfer this solution to a chromatographic column, prepared by pouring 10 mL of strongly acidic ion-exchange resin (H type) (424 – 600  $\mu\text{m}$  in particle diameter) into a chromatographic tube, 9 mm in inside diameter and 160 mm in height, and elute at the rate of about 4 mL per minute. Wash the chromatographic column with 50 mL of water at the rate of about 4 mL per minute, and wash again with 100 mL of water in the same manner. Combine the washings with the eluate, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium hydroxide VS  
 = 17.42 mg of  $\text{C}_5\text{H}_{11}\text{NaO}_3\text{S}$

**Sodium perchlorate** See sodium perchlorate monohydrate.

**Sodium perchlorate monohydrate**  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$   
 [K 8227, Special class]

**Sodium periodate**  $\text{NaIO}_4$  [K 8256, Special class]

**Sodium periodate TS** Dissolve 60.0 g of sodium periodate in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. If the solution is not clear, filter this through a glass-filter. Keep in a light-resistant vessel.

**Sodium peroxide**  $\text{Na}_2\text{O}_2$  [K 8231, Special class]

**Sodium *p*-phenol sulfonate** See sodium *p*-phenol sulfonate dihydrate.

**Sodium *p*-phenol sulfonate dihydrate**  $\text{C}_6\text{H}_5\text{O}_4\text{NaS} \cdot 2\text{H}_2\text{O}$   
 White to light yellow, crystals or crystalline powder, having a specific odor.

**Identification** (1) To 10 mL 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 Ultraviolet-visible Spectrophotometry <2.24>: 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 25 mL 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 50 mL 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  $\mu\text{m}$  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 <2.50> 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 50 mL of water and titrate with 0.1 mol/L sodium hydroxide VS, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
 = 23.22 mg of  $\text{C}_6\text{H}_5\text{O}_4\text{NaS} \cdot 2\text{H}_2\text{O}$

**Sodium phosphate** See trisodium phosphate dodecahydrate.

**Sodium phosphate TS** Dissolve 5.68 g of disodium hydrogen phosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

#### 0.1 mol/L Sodium phosphate buffer solution, pH 7.0

Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL. Add to this solution to a 500 mL solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in water until the pH becomes 7.0.

**Sodium pyruvate** Prepared for microbial test.

**Sodium salicylate**  $\text{HOC}_6\text{H}_4\text{COONa}$  [K 8397, Special class]

**Sodium salicylate-sodium hydroxide TS** Dissolve 1 g of sodium salicylate in 0.01 mol/L sodium hydroxide VS to make 100 mL.

**Sodium selenite**  $\text{Na}_2\text{SeO}_3$  A white crystalline powder.

**Identification**—(1) Dissolve 1 g of sodium selenite in 100 mL of water, and use this as the sample solution. To 10 mL of the sample solution add 2 mL 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.

Recrystallize 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 Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about  $1236\text{ cm}^{-1}$ ,  $1192\text{ cm}^{-1}$ ,  $1136\text{ cm}^{-1}$ ,  $1052\text{ cm}^{-1}$ ,  $844\text{ cm}^{-1}$  and  $688\text{ cm}^{-1}$ .

**Purity**—Perform the test with  $10\ \mu\text{L}$  of a solution of sodium *p*-styrenesulfonate (1 in 1000) as directed in the Assay under Panipenem: Any obstructive peaks for determination of panipenem are not observed.

**Sodium sulfate** See sodium sulfate decahydrate.

**Sodium sulfate, anhydrous**  $Na_2SO_4$  [K 8987, Special class]

**Sodium sulfate decahydrate**  $Na_2SO_4 \cdot 10H_2O$  [K 8986, Special class]

**Sodium sulfide** See sodium sulfide enneahydrate.

**Sodium sulfide enneahydrate**  $Na_2S \cdot 9H_2O$  [K 8949, Special class]

**Sodium sulfide TS** Dissolve 5 g of sodium sulfide enneahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL 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**  $Na_2SO_3$  [K 8061, Sodium sulfite, Special class]

**Sodium sulfite heptahydrate**  $Na_2SO_3 \cdot 7H_2O$  [K 8060, Special class]

**1 mol/L Sodium sulfite TS** Dissolve 1.26 g of anhydrous sodium sulfite in water to make 10 mL.

**Sodium sulfite-sodium dihydrogen phosphate TS** Mix 1.5 mL of a solution dissolved 1.26 g of anhydrous sodium sulfite in 100 mL of water and 98.5 mL of a solution dissolved 1.56 g of sodium dihydrogen phosphate dihydrate in 100 mL of water. Prepare before use.

**Sodium tartrate** See sodium tartrate dihydrate.

**Sodium tartrate dihydrate**  $C_4H_4Na_2O_6 \cdot 2H_2O$  [K 8540, Special class]

**Sodium tauroursodeoxycholate for thin-layer chromatography**  $C_{26}H_{44}NNaO_6S \cdot xH_2O$  White to pale brown crystalline powder or powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

**Identification**—Determine the infrared absorption spectrum of sodium tauroursodeoxycholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits the absorption at the wave numbers of about  $2940\text{ cm}^{-1}$ ,  $1600\text{ cm}^{-1}$ ,  $1410\text{ cm}^{-1}$ ,  $1305\text{ cm}^{-1}$ ,  $1195\text{ cm}^{-1}$ ,  $1080\text{ cm}^{-1}$ ,

$1045\text{ cm}^{-1}$ ,  $980\text{ cm}^{-1}$ ,  $950\text{ cm}^{-1}$ ,  $910\text{ cm}^{-1}$  and  $860\text{ cm}^{-1}$ .

**Optical rotation** <2.49>  $[\alpha]_D^{20}$ :  $+40 - +50^\circ$  (40 mg, methanol, 20 mL, 100 mm).

**Purity** Related substances—Dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Perform the test with  $5\ \mu\text{L}$  each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot at the *R<sub>f</sub>* value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

**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 800 mL of freshly boiled and cooled water, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Sodium tetraborate decahydrate**  $Na_2B_4O_7 \cdot 10H_2O$  [K 8866, Special class]

**Sodium tetraborate decahydrate for pH determination** [K 8866, for pH standard solution]

**Sodium tetraborate-sulfuric acid TS** To 9.5 g of sodium tetraborate decahydrate add 1000 mL of purified sulfuric acid, and dissolve by shaking for a night.

**Sodium tetraphenylborate**  $(C_6H_5)_4BNa$  [K 9521]

**Sodium thioglycolate**  $HSCH_2COONa$  A white powder, having a characteristic odor.

**Identification** (1) To a solution (1 in 10) add 1 drop each of ammonia solution (28) and iron (III) chloride TS: a dark red-purple color appears.

(2) Perform the test as directed under Flame Coloration Test (1) <1.04>: a yellow color appears.

**Purity** Clarity and color of solution—Dissolve 1 g in 10 mL of water: the solution is clear and colorless.

**Sodium thiosulfate** See sodium thiosulfate pentahydrate.

**Sodium thiosulfate pentahydrate**  $Na_2S_2O_3 \cdot 5H_2O$  [K 8637, 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 1000 mL (0.1 mol/L).

**Sodium toluenesulfonchloramide trihydrate**  $C_7H_7ClNNaO_2S \cdot 3H_2O$  [K 8318, Sodium *p*-toluenesulfonchloramide trihydrate, Special class]

**Sodium toluenesulfonchloramide TS** Dissolve 1 g of sodium toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare before use.

**Sodium tridecanesulfonate**  $C_{13}H_{27}SO_3Na$  White, crystals or powder.

**Purity** Absorbance—Dissolve 1.43 g of sodium tridecanesulfonate in 1000 mL of water, and perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 230 nm and 245 nm are not more than

0.05 and 0.01, respectively.

**Sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy**  $(\text{CH}_3)_3\text{SiCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$   
Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 3-trimethylsilylpropionate- $d_4$  for nuclear magnetic resonance spectroscopy**  $(\text{CH}_3)_3\text{SiCD}_2\text{CD}_2\text{COONa}$   
Prepared for nuclear magnetic resonance spectroscopy.

**Sodium 2,4,6-trinitrobenzenesulfonate dihydrate**  
 $\text{C}_6\text{H}_2\text{N}_3\text{NaO}_9\text{S}\cdot 2\text{H}_2\text{O}$  White or pale yellowish crystals or powder.

**Sodium tungstate** See sodium tungstate (VI) dihydrate.

**Sodium tungstate (VI) dihydrate**  $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$   
[K 8612, Special class]

**Sodium valproate for assay**  $\text{C}_8\text{H}_{15}\text{NaO}_2$  [Same as the monograph Sodium Valproate. When dried, it contains not less than 99.0% of sodium valproate ( $\text{C}_8\text{H}_{15}\text{NaO}_2$ ).]

**Solid plates** Dilute anti-*E. coli* protein antibody stock solution by adding 0.2 mol/L Tris hydrochloride buffer, pH 7.4, to a concentration of about 0.02 mg/mL. Add exactly 0.1 mL of this solution to each well in the microplates, cover with plate seal, and then shake gently. Centrifuge for 2 minutes if some solution sticks to the top of the microplate or elsewhere. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS (pH 7.4) to make the wash solution. After leaving the microplates for 16 to 24 hours at a constant temperature of about 25°C, remove the solution in each well by aspiration, add 0.25 mL of the wash solution, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times for each well using 0.25 mL of the wash solution. Add 0.25 mL of the block buffer solution to each well, gently shake, and let stand for 16 to 24 hours at a constant temperature of about 25°C to make solid plates. When using, remove the solution from the wells by aspiration, add 0.25 mL of the wash solution to each well, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times using 0.25 mL of the wash solution.

**Soluble starch** See starch, soluble.

**Soluble starch TS** Triturate 1 g of soluble starch in 10 mL of cooled water, pour gradually into 90 mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

**Sorbitan sesquioleate** [Same as the namesake monograph]

**D-Sorbitol** [Same as the namesake monograph]

**D-Sorbitol for gas chromatography** Prepared for gas chromatography.

**Soybean-casein digest medium** See Sterility Test <4.06>.

**Soybean oil** [Same as the namesake monograph]

**Soybean peptone** See peptone, soybean.

**Stacking gel for celmoleukin** In 0.5 mol/L Tris buffer solution, pH 6.8, prepare stacking the gel using ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine so the acrylamide concentration is 5.2% and the sodium lauryl sulfate concentration is 0.1%.

**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** [K 8658, Special class]

**Starch-sodium chloride TS** Saturate starch TS with sodium chloride. Use within 5 to 6 days.

**Starch, soluble** [K 8659, Special class]

**Starch TS** Triturate 1 g of starch with 10 mL of cold water, and pour the mixture slowly, with constant stirring, into 200 mL of boiling water. Boil the mixture until a thin, translucent fluid is obtained. Allow to settle, and use the supernatant liquid. Prepare before use.

**Stearic acid for gas chromatography**  $\text{C}_{18}\text{H}_{36}\text{O}_2$   
[K 8585, Special class]

**Stearyl alcohol** [Same as the namesake monograph]

**Sterile purified water** [Same as the monograph Sterile Purified Water in Containers. It is not necessary to confirm if they meet all of the requirement, provided that they are confirmed to be suitable for the purpose of the relevant test.]

**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).

**Strongly acidic ion exchange resin** Contains strong acid ion exchange residues. Particle diameter is about 100  $\mu\text{m}$ .

**Strongly basic ion exchange resin** Contains strong basic ion exchange residues. Particle diameter is about 100  $\mu\text{m}$ .

**Strontium TS** Dissolve 76.5 g of strontium chloride in water to make exactly 500 mL. Pipet 20 mL of this solution, and add water to make exactly 1000 mL (1000 ppm).

**Strontium chloride** See strontium chloride hexahydrate.

**Strontium chloride hexahydrate**  $\text{SrCl}_2\cdot 6\text{H}_2\text{O}$  [K 8132, Special class]

**Strychnine nitrate for assay**  $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2\cdot \text{HNO}_3$  To 1 g of strychnine nitrate add 14 mL of water and about 10 mg of active carbon, heat in a water bath for 10 minutes, filter while hot, cool the filtrate quickly to form crystals, and filter the crystals. Add 8 mL of water to the crystals, dissolve by heating in a water bath, filter while hot, cool quickly, and filter the crystals formed. Repeat this procedure with 8 mL of water, and dry the crystals in a desiccator (in vacuum, silica gel) for 24 hours. Colorless or white crystals or crystalline powder. Sparingly soluble in water, in glycerin and in chloroform, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Purity** Related substances—Dissolve 35 mg of strychnine nitrate for assay in 100 mL of the mobile phase and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and

use this solution as the standard solution (1). Perform the test with exactly 20  $\mu\text{L}$  each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than strychnine from the sample solution is not larger than the peak area of strychnine from the standard solution (1).

#### Operating conditions

Proceed the operating conditions in the Assay under Nux Vomica except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 40 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of strychnine obtained from 20  $\mu\text{L}$  of the standard solution (2) can be measured by the automatic integration method and the peak height of strychnine from 20  $\mu\text{L}$  of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of strychnine beginning after the solvent peak.

Loss on drying <2.41>: not more than 0.5% (0.2 g, 105°C, 3 hours).

Content: not less than 99.0% calculated on the dried basis. Assay—Dissolve about 0.5 g of strychnine nitrate for assay, accurately weighed, in 40 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), heat if necessary, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 39.74 mg of  $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 \cdot \text{HNO}_3$

**Styrene**  $\text{C}_8\text{H}_8$  Colorless, clear liquid.

Specific gravity <2.56>  $d$ : 0.902 – 0.910

Purity—Perform the test with 1  $\mu\text{L}$  of styrene as directed under Gas Chromatography <2.02> 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: it shows the purity of not less than 99%.

#### Operating conditions

Detector: 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  $\mu\text{m}$  in particle diameter) coated with polyethylene glycol 20 M 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, beginning after the solvent peak.

**Styrene-maleic acid alternating copolymer partial butyl ester** A copolymer of styrene and maleic anhydride, polymerized using cumene as solvent and added 1-butanol or water to the maleic anhydride groups. Average molecular mass: about 1600. A white or pale yellowish white powder.

**Identification**—Dissolve 5 mg of the substance to be examined in sodium hydrogen carbonate solution (1 in 15) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 256 nm and 260 nm, and a shoulder between 251 nm and 256 nm.

Absorbance <2.24>  $E_{1\text{cm}}^{1\%}$  (258 nm): 6.3 – 7.3 [5 mg calculated on the anhydrous basis, sodium hydrogen carbonate solution (1 in 15), 10 mL]

Purity—Proceed as directed in the Purity (3) under Zinostatin Stimalamer, with the exception of without using of (iii) Standard solution, and changing (iv) Sample solution, (v) Procedure and (vii) Determination as follows:

(iv) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 20 mL.

(v) Procedure Mount the gel in the electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the top reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100  $\mu\text{L}$  of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached at 5 cm from the upper end of the gel.

(vii) Determination Determine the peak area,  $A_T$ , of styrene-maleic acid alternating copolymer partial butyl ester and the total area,  $A$ , of the peaks other than styrene-maleic acid alternating copolymer partial butyl ester, based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of styrene-maleic acid alternating copolymer partial butyl ester by the following formula: not less than 98.0%.

Amount (%) of styrene-maleic acid alternating copolymer partial butyl ester  
=  $A_T / (A_T + A) \times 100$

Water <2.48>: Not more than 10.0% (10 mg, coulometric titration).

**Substrate buffer for celmoleukin** Dissolve 32.4 g of tripotassium citrate monohydrate in water to make 1000 mL, and add 1 mol/L citric acid TS for buffer solution to adjust the pH to 5.5. To 100 mL of this solution add and dissolve 0.44 g of *o*-phenylenediamine and then 60  $\mu\text{L}$  of hydrogen peroxide (30). Prepare at the time of use.

**Substrate TS for lysozyme hydrochloride** To a suitable amount of dried cells of *Micrococcus luteus* add a suitable amount of phosphate buffer solution, pH 6.2, gently shake to make a suspension, and add the substrate cells or the same buffer solution so that the absorbance of the suspension at 640 nm is about 0.65. Prepare before use.

**Substrate TS for peroxidase determination** Dissolve 0.195 mL of hydrogen peroxidase (30), 8.38 g of disodium hydrogen phosphate dodecahydrate and 1.41 g of citric acid monohydrate in water to make 300 mL. To 15 mL of this solution add 13 mg of *o*-phenylenediamine dihydrochloride before use.

**Substrate TS for kallidinogenase assay (1)** Dissolve an

appropriate amount of H-D-valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to prepare a solution containing 1 mg of H-D-valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride in 5 mL.

**Substrate TS for kallidinogenase assay (2)** Dissolve 17.7 mg of *N*- $\alpha$ -benzoyl-L-arginine ethyl ester hydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to make 100 mL.

**Substrate TS for kallidinogenase assay (3)** Suspend 0.6 g of milk casein purified by the Hammerstein's method in 80 mL of 0.05 mol/L sodium hydrogen phosphate TS, and dissolve by warming at 65°C for 20 minutes. After cooling, adjust to pH 8.0 with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 100 mL. Prepare before use.

**Substrate TS for kallidinogenase assay (4)** Dissolve 25 mg of H-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 28.8 mL of water.

**Succinic acid, anhydrous** C<sub>4</sub>H<sub>4</sub>O<sub>3</sub> 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 <1.03>: not more than 0.005%.

(2) Iron <1.10>: not more than 0.001%.

*Residue on ignition* <2.44>: not more than 0.1% (1 g).

*Content*: not less than 98.0%. *Assay*—Dissolve about 1 g of anhydrous succinic acid, accurately weighed, in 50 mL of water by warming, cool, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 50.04 mg of C<sub>4</sub>H<sub>4</sub>O<sub>3</sub>.

**Sucrose** C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> [Same as the monograph Sucrose]

**Sudan III** C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>O 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* <2.60>: 170 – 190°C

**Sudan III TS** Dissolve 0.01 g of sudan III in 5 mL of ethanol (95), filter, and add 5 mL of glycerin to the filtrate. Prepare before use.

**Sulbactam sodium for sulbactam penicillamine** C<sub>8</sub>H<sub>10</sub>NNaO<sub>3</sub>S 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 Infrared Spectrophotometry <2.25>: it exhibits the absorption at the wave numbers of about 1780 cm<sup>-1</sup>, 1600 cm<sup>-1</sup>, 1410 cm<sup>-1</sup>, 1400 cm<sup>-1</sup>, 1320 cm<sup>-1</sup>, 1300 cm<sup>-1</sup>, 1200 cm<sup>-1</sup> and 1130 cm<sup>-1</sup>.

*Water* <2.48>: not more than 1.0% (0.5 g).

*Content*: not less than 875  $\mu$ g per mg, calculated on the anhydrous basis. *Assay*—Weigh accurately an amount of sulbactam sodium for sulbactam penicillamine and Sulbactam RS, equivalent to about 0.10 g (potency), dissolve each in a suitable volume of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to

make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10  $\mu$ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios,  $Q_T$  and  $Q_S$ , of the peak area of sulbactam to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of sulbactam (C}_8\text{H}_{11}\text{NO}_5\text{S)} \\ = M_S \times Q_T/Q_S \times 1000 \end{aligned}$$

$M_S$ : amount [mg (potency)] of Sulbactam RS

*Internal standard solution*—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

*Operating conditions*

*Detector*: Ultraviolet absorption photometer (wavelength: 220 nm)

*Column*: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10  $\mu$ m in particle diameter).

*Column temperature*: A constant temperature of about 35°C.

*Mobile phase*: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

*Flow rate*: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

*System suitability*

*System performance*: When the procedure is run with 10  $\mu$ L of the standard solution according to the above operating 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  $\mu$ L of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 2.0%.

**Sulfamic acid (standard reagent)** See amido sulfuric acid (standard reagent).

**Sulfanilamide** H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NH<sub>2</sub> [K 9066, Special class]

**Sulfanilamide for titration of diazotization**  
H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NH<sub>2</sub> [K 9066, For titration of diazotization]

**Sulfanilic acid** H<sub>2</sub>NC<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>H [K 8586, Special class]

**Sulfathiazole** C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub> White crystalline powder.

*Melting point* <2.60>: 200 – 204°C

**Sulfosalicylic acid** See 5-sulfosalicylic acid dihydrate.

**5-Sulfosalicylic acid dihydrate** C<sub>7</sub>H<sub>6</sub>O<sub>6</sub>S.2H<sub>2</sub>O  
[K 8589, Special class]

**Sulfosalicylic acid TS** Dissolve 5 g of 5-sulfosalicylic acid dihydrate in water to make 100 mL.

**Sulfur** S [K 8088, Special class]

**Sulfur dioxide** SO<sub>2</sub> Prepare by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

**Sulfuric acid** H<sub>2</sub>SO<sub>4</sub> [K 8951, Special class]

**Sulfuric acid, dilute** Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make

100 mL (10%).

**Sulfuric acid-ethanol TS** With stirring, add slowly 3 mL of sulfuric acid to 1000 mL of ethanol (99.5), and cool.

**Sulfuric acid for readily carbonizable substances** To sulfuric acid, the content of which has previously been determined by the following method, add water cautiously, and adjust the final concentration to 94.5% to 95.5% of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). When the concentration is changed owing to absorption of water during storage, prepare freshly.

*Assay*—Weigh accurately about 2 g of sulfuric acid in a glass-stoppered flask rapidly, add 30 mL of water, cool, and titrate <2.50> the solution with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS  
= 49.04 mg of H<sub>2</sub>SO<sub>4</sub>

**Sulfuric acid, fuming** H<sub>2</sub>SO<sub>4</sub>.nSO<sub>3</sub> [K 8741, Special class]

**Sulfuric acid-hexane-methanol TS** To 230 mL of a mixture of hexane and methanol (1:3) add cautiously 2 mL of sulfuric acid.

**Sulfuric acid-methanol TS** Prepare carefully by adding 60 mL of sulfuric acid to 40 mL of methanol.

**Sulfuric acid-methanol TS, 0.05 mol/L** Add gradually 3 mL of sulfuric acid to 1000 mL of methanol, while stirring, and allow to cool.

**Sulfuric acid-monobasic sodium phosphate TS** See sulfuric acid-sodium dihydrogenphosphate TS.

**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 dihydrogenphosphate TS** Add 6.8 mL of sulfuric acid to 500 mL of water, then dissolve 50 g of sodium dihydrogenphosphate dihydrate in this solution, and add water to make 1000 mL.

**Sulfuric acid-sodium hydroxide TS** With stirring add slowly 120 mL of sulfuric acid to 1000 mL of water, and cool (solution A). Dissolve 88.0 g of sodium hydroxide in 1000 mL of freshly boiled and cooled water (solution B). Mix equal volumes of solution A and solution B.

**Sulfuric acid TS** Cautiously add 1 volume of sulfuric acid to 2 volumes of water, and while warming on a water bath add dropwise potassium permanganate TS until a pale red color of the solution remains.

**0.05 mol/L Sulfuric acid TS** Dilute 100 mL of 0.5 mol/L sulfuric acid TS with water to make 1000 mL.

**0.25 mol/L Sulfuric acid TS** With stirring, add slowly 15 mL of sulfuric acid to 1000 mL of water, then cool.

**0.5 mol/L Sulfuric acid TS** With stirring, add slowly 30 mL of sulfuric acid to 1000 mL of water, then cool.

**1 mol/L Sulfuric acid TS** Add 60 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

**2 mol/L Sulfuric acid TS** To 1000 mL of water add gradually 120 mL of sulfuric acid with stirring, and cool.

**5 mol/L Sulfuric acid TS** Add 300 mL of sulfuric acid in

1000 mL of water slowly with stirring, then allow to cool.

**Sulfurous acid** See sulfurous acid solution.

**Sulfurous acid solution** A clear and colorless liquid containing more than 5% of SO<sub>2</sub>, having a pungent odor. Specific gravity: about 1.03 g/mL.

*Identification*—To 1 mL of iodine TS add 20 mL of water, and add 1 mL of sulfurous acid solution: the color of the solution disappears, and this solution forms a white precipitate upon addition of 1 mL of barium chloride TS.

Preserve at a cold place.

**Sulpiride for assay** C<sub>15</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S [Same as the monograph Sulpiride. When dried, it contains not less than 99.0% of sulpiride (C<sub>15</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S).]

**Sulpyrine** See sulpyrine hydrate.

**Sulpyrine for assay** See sulpyrine hydrate for assay.

**Sulpyrine hydrate** C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>NaO<sub>4</sub>S.H<sub>2</sub>O [Same as the namesake monograph]

**Sulpyrine hydrate for assay** C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>NaO<sub>4</sub>S.H<sub>2</sub>O [Same as the monograph Sulpyrine Hydrate. Calculated on the dried basis, it contains not less than 99.0% of sulpyrine (C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>NaO<sub>4</sub>S).]

**Suxamethonium chloride for thin-layer chromatography** See suxamethonium chloride hydrate for thin-layer chromatography.

**Suxamethonium chloride hydrate for thin-layer chromatography** C<sub>14</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>.2H<sub>2</sub>O [Same as the monograph Suxamethonium Chloride Hydrate]

**Swertia herb** [Same as the namesake monograph]

**Swertiamarin for thin-layer chromatography** C<sub>16</sub>H<sub>22</sub>O<sub>10</sub> White, practically tasteless powder.

*Melting point* <2.60>: 113 – 114°C

*Purity* Related substances—Dissolve 2.0 mg of swertiamarin for thin-layer chromatography in exactly 1 mL of ethanol (95), and perform the test with 20 μL of this solution as directed in the Identification under Swertia Herb: any spot other than the principal spot at the R<sub>f</sub> value of about 0.5 does not appear.

**Synthetic zeolite for drying** A mixture of 6(Na<sub>2</sub>O).6(Al<sub>2</sub>O<sub>3</sub>).12(SiO<sub>2</sub>) and 6(K<sub>2</sub>O).6(Al<sub>2</sub>O<sub>3</sub>).12(SiO<sub>2</sub>) 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 m<sup>2</sup> per g.

*Loss on ignition* <2.43>: not more than 2.0% [2 g, 550 – 600°C, 4 hours, allow to stand in a desiccator (phosphorus (V) oxide).]

**Talc** [Same as the namesake monograph]

**Tamsulosin hydrochloride** C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>S.HCl [Same as the namesake monograph]

**Tamsulosin hydrochloride for assay** C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>S.HCl [Same as the monograph Tamsulosin Hydrochloride. When dried, it contains not less than 99.0% of tamsulosin hydrochloride (C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>S.HCl).]

**Tannic acid** [Same as the namesake monograph]

**Tannic acid TS** Dissolve 1 g of tannic acid in 1 mL of ethanol (95), and add water to make 10 mL. Prepare before use.

**Tartaric acid** See L-tartaric acid.

**L-Tartaric acid**  $C_4H_6O_6$  [K 8532, L(+)-Tartaric acid, Special class].

**Tartrate buffer solution, pH 3.0** Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartrate dihydrate in water to make 1000 mL.

**Taurine**  $H_2NCH_2CH_2SO_3H$  White crystals or crystalline powder.

*Contents:* not less than 95.0%. Assay—Weigh accurately about 0.2 g, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 12.52 mg of  $C_2H_7NO_3S$

**Temocapril hydrochloride for assay**  $C_{23}H_{28}N_2O_5S_2.HCl$  [Same as the monograph Temocapril Hydrochloride. It contains not less than 99.5% of temocapril hydrochloride ( $C_{23}H_{28}N_2O_5S_2.HCl$ : 513.07), calculated on the anhydrous basis.]

**Terbinafine hydrochloride for assay**  $C_{21}H_{25}N.HCl$  [Same as the monograph Terbinafine Hydrochloride]

**Terephthalic acid**  $C_6H_4(COOH)_2$  White crystals or crystalline powder. Slightly soluble in ethanol (95), and practically insoluble in water and ether.

*Residue on ignition* <2.44>: 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 50 mL of 1 mol/L sodium hydroxide VS, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS  
= 83.07 mg of  $C_8H_6O_4$

**Terphenyl**  $C_{18}H_{14}$  White crystalline powder.

*Identification*—Determine the absorption spectrum of a solution of terphenyl in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 276 and 280 nm.

*Melting point* <2.60>: 208 – 213°C

**p-Terphenyl** See terphenyl.

**Test bacteria inoculation medium for teceleukin** Dissolve 6.0 g of peptone, 3.0 g of yeast extract, 1.5 g of meat extract, 1.0 g of glucose, and 13.0 to 20.0 g of agar in 1000 mL of water and sterilize. The pH is 6.5 to 6.6.

**Test bacteria inoculation medium slant for teceleukin** Sterilized slant culture obtained by adding approximately 9 mL of bacteria inoculation medium for teceleukin to a test tube with an inside diameter of 16 mm.

**Testosterone**  $C_{19}H_{28}O_2$  White crystals or crystalline powder.

*Identification*—Determine the infrared absorption spec-

trum of testosterone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits the absorption at the wave numbers of about 3530  $cm^{-1}$ , 3380  $cm^{-1}$ , 1612  $cm^{-1}$ , 1233  $cm^{-1}$ , 1067  $cm^{-1}$  and 1056  $cm^{-1}$ .

**Testosterone propionate**  $C_{22}H_{32}O_3$  [Same as the name-sake monograph]

**Tetrabromophenolphthalein ethyl ester potassium salt**  $C_{22}H_{13}O_4Br_4K$  [K 9042, Special class]

**Tetrabromophenolphthalein ethyl ester TS** Dissolve 0.1 g of tetrabromophenolphthalein ethyl ester potassium salt in acetic acid (100) to make 100 mL. Prepare before use.

**Tetra-n-butylammonium bromide**  $[CH_3(CH_2)_3]_4NBr$  White, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 101 – 105°C

*Purity* Clarity and color of solution—Dissolve 1.0 g of tetra-n-butylammonium bromide in 20 mL of water: the solution is clear and colorless.

*Content:* not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-n-butylammonium bromide, accurately weighed, in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 32.24 mg of  $C_{16}H_{36}NBr$

**Tetra-n-butylammonium chloride**  $C_{16}H_{36}ClN$  White crystals, and it is deliquescent.

*Water* <2.48>: not more than 6.0% (0.1 g).

*Content:* not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.25 g of tetra-n-butylammonium chloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS  
= 27.79 mg of  $C_{16}H_{36}ClN$

**Tetrabutylammonium hydrogensulfate**  $C_{16}H_{37}NO_4S$  White crystalline powder.

*Content:* not less than 98.0%. Assay—Weigh accurately about 0.7 g of tetrabutylammonium hydrogensulfate, dissolve in 100 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromocresol green-methyl red TS).

Each mL of 0.1 mol/L sodium hydroxide VS  
= 33.95 mg of  $C_{16}H_{37}NO_4S$

**40% Tetrabutylammonium hydroxide TS** A solution containing 40 g/dL of tetrabutylammonium hydroxide [ $(C_4H_9)_4NOH$ : 259.47].

*Content:* 36 – 44 g/dL. Assay—Pipet 10 mL of 40% tetrabutylammonium hydroxide TS, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS  
= 259.5 mg of  $C_{16}H_{37}NO$

**0.005 mol/L Tetrabutylammonium hydroxide TS** To 10 mL of tetrabutylammonium hydroxide TS add 700 mL of

water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

**Tetrabutylammonium hydroxide-methanol TS** Methanol solution containing 25 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47]. Colorless to pale yellow solution, having an ammonium-like odor.

*Content:* 22.5 – 27.5 g/dL. Assay—Pipet 15 mL of tetrabutylammonium hydroxide-methanol TS and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS  
= 259.5 mg of C<sub>16</sub>H<sub>37</sub>NO

**10% Tetrabutylammonium hydroxide-methanol TS** A methanol solution containing 10 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Content:* 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of 10% tetrabutylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 25.95 mg of C<sub>16</sub>H<sub>37</sub>NO

**Tetrabutylammonium hydroxide TS** A solution containing 13 g/dL of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH: 259.47].

*Content:* 11.7 – 14.3 g/dL. Assay—Pipet a quantity, equivalent to about 0.3 g of tetrabutylammonium hydroxide [(C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NOH], transfer to a glass-stoppered flask containing 15 mL of water, accurately weighed, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 25.95 mg of C<sub>16</sub>H<sub>37</sub>NO

**Tetrabutylammonium phosphate** (C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NH<sub>2</sub>PO<sub>4</sub> White powder. It is soluble in water.

*Content:* not less than 97.0%. Assay—Weigh accurately 1.5 g of tetrabutylammonium phosphate, dissolve in 80 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS  
= 169.7 mg of (C<sub>4</sub>H<sub>9</sub>)<sub>4</sub>NH<sub>2</sub>PO<sub>4</sub>

**Tetracycline** C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub> 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 μg (potency) per mg. Assay—Proceed as directed in the Assay under Tetracycline Hydrochloride. However, use the following formula.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of tetracycline (C}_{22}\text{H}_{24}\text{N}_2\text{O}_8) \\ = M_S \times (A_T/A_S) \times 1000$$

M<sub>S</sub>: Amount [mg (potency)] of Tetracycline Hydrochloride RS

**Tetracycline Hydrochloride** C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>·HCl Yellow, crystals or crystalline powder.

*Purity* Related substances—Dissolve 20 mg of tetracycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pro-

ceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than tetracycline is not more than 10%.

**Tetradecyl trimethylammonium bromide**

CH<sub>3</sub>(CH<sub>2</sub>)<sub>13</sub>N(CH<sub>3</sub>)<sub>3</sub>Br A white powder.

*Purity* Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless.

*Content:* not less than 98.0%. Assay—Weigh accurately about 0.5 g of tetradecyl trimethylammonium bromide, dissolve in 100 mL of water, add 5 mL of a mixture of water and nitric acid (2:1), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 33.64 mg of C<sub>17</sub>H<sub>38</sub>NBr

**Tetraethylammonium hydroxide TS** A solution containing 10% of tetraethylammonium hydroxide [(C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>NOH: 147.26]. A clear, colorless liquid, having a strong ammonia odor. It is a strong basic and easily absorbs carbon dioxide from the air.

*Content:* 10.0 – 11.0% Assay—Weigh accurately about 3 g in a glass-stoppered flask containing 15 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS  
= 14.73 mg of C<sub>8</sub>H<sub>21</sub>NO

**Tetra-*n*-heptylammonium bromide** [CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>]<sub>4</sub>NBr

White, crystals or crystalline powder, having a slight, characteristic odor.

*Melting point* <2.60>: 87 – 89°C

*Content:* not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-*n*-heptylammonium bromide, accurately weighed, in 50 mL of diluted acetonitrile (3 in 5), and 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 49.07 mg C<sub>28</sub>H<sub>60</sub>NBr

**Tetrahydrofuran** CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>O [K 9705, Special class]

**Tetrahydrofuran for gas chromatography** Use tetrahydrofuran prepared by distilling with iron (II) sulfate heptahydrate.

*Storage*—Preserve in containers, in which the air has been displaced by nitrogen, in a dark, cold place.

**Tetrahydrofuran for liquid chromatography** C<sub>4</sub>H<sub>8</sub>O

Clear and colorless liquid.

*Refractive index* <2.45> n<sub>D</sub><sup>20</sup>: 1.406 – 1.409

*Density* <2.56> 0.884 – 0.889 g/mL (20°C)

*Purity* Ultraviolet absorbing substances—Determine the absorption spectrum of tetrahydrofuran for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbances at

240 nm, 254 nm, 280 nm, 290 nm, and between 300 nm and 400 nm are not more than 0.35, 0.20, 0.05, 0.02 and 0.01, respectively.

**Peroxide**—Perform the test according to the method described in JIS K 9705: not more than 0.01%.

**Tetrahydroxyquinone**  $C_6H_4O_6$  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.

**Tetrakis(hydroxypropyl)ethylenediamine for gas chromatography** Prepared for gas chromatography.

**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 5 mL of water. Add a slight excess of 1 mol/L hydrochloric acid TS (about 4 mL), 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  $\pm 0.2\%$  of that found in the Assay.

**Residue on evaporation:** not more than 0.02% (5 mL, 105°C, 1 hour).

**Content:** not less than 98% of the labeled amount. **Assay**—Accurately weigh a glass-stoppered flask containing about 15 mL of water. Add a quantity of the solution, equivalent to about 0.2 g of tetramethylammonium hydroxide  $[(CH_3)_4NOH]$ , weigh again, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 9.115 mg of  $C_4H_{13}NO$

**Tetramethylammonium hydroxide-methanol TS** A methanol solution containing of 10 g/dL of tetramethylammonium hydroxide  $[(CH_3)_4NOH]$ : 91.15]

**Content:** 9.0 – 11.0 g/dL. **Assay**—Pipet 2 mL of tetramethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS  
= 9.115 mg of  $C_4H_{13}NO$

**Tetramethylammonium hydroxide TS** Pipet 15 mL of tetramethylammonium hydroxide, and add dehydrated ethanol (99.5) to make exactly 100 mL.

**Tetramethylammonium hydroxide TS, pH 5.5** To 10 mL of tetramethylammonium hydroxide add 990 mL of water, and adjust the pH to 5.5 with diluted phosphoric acid (1 in 10).

**3,3',5,5'-Tetramethylbenzidine dihydrochlorate dihydrate**  $C_{16}H_{22}Cl_2N_2 \cdot H_2O$  White to slightly reddish-white crystalline powder.

***N,N,N',N'*-Tetramethylethylenediamine**  
 $(CH_3)_2NCH_2CH_2N(CH_3)_2$  Pale yellow clear liquid.

**Specific gravity** <2.56>  $d_4^{20}$ : 0.774 – 0.799

**Content:** not less than 99.0%.

**Tetramethylsilane for nuclear magnetic resonance spectroscopy**  $(CH_3)_4Si$  Prepared for nuclear magnetic resonance spectroscopy.

**Tetra-*n*-pentylammonium bromide**  $[CH_3(CH_2)_4]_4NBr$  White, crystals or crystalline powder. It is hygroscopic.

**Melting point** <2.60>: 100 – 101°C

**Tetraphenylboron potassium TS** Add 1 mL of acetic acid (31) to a solution of potassium biphthalate (1 in 500), then to this solution add 20 mL of a solution of tetraphenylboron sodium (7 in 1000), shake well, 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 100 mL 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 30 mL of the filtrate.

**Tetraphenylboron sodium** See sodium tetraphenylborate.

**Tetra-*n*-propylammonium bromide**  $[CH_3CH_2CH_2]_4NBr$  White, crystals or crystalline powder.

**Purity** Clarity and color of solution—Dissolve 1.0 g of tetra-*n*-propylammonium bromide in 20 mL of water: the solution is clear and colorless.

**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.4 g of tetra-*n*-propylammonium bromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking strongly (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS  
= 26.63 mg of  $C_{12}H_{28}NBr$

**Theophylline**  $C_7H_8N_4O_2$  White powder. Slightly soluble in water.

**Melting point** <2.60>: 269 – 274°C

**Purity** Caffeine, theobromine or paraxanthine—To 0.20 g of theophylline add 5 mL of potassium hydroxide TS or 5 mL of ammonia TS: each solution is clear.

**Loss on drying** <2.41>: 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 40 mL of *N,N*-dimethylformamide, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium methoxide VS  
= 18.02 mg of  $C_7H_8N_4O_2$

**Theophylline for assay**  $C_7H_8N_4O_2$  [Same as the monograph Theophylline meeting the following additional specifications.]

**Purity** Related substances—Dissolve 50 mg of theophylline for assay in water to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20  $\mu$ L of the

sample solution and standard solution as directed under Liquid Chromatography <2.01>. Determine each peak from both solutions by the automatic integration method: the total area of peaks other than the peak of theophylline obtained from the sample solution is not larger than the peak area of theophylline from the standard solution.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 270 nm).

**Column:** A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

**Flow rate:** Adjust the flow rate so that the retention time of theophylline is about 10 minutes.

**Time span of measurement:** About 3 times as long as the retention time of theophylline.

#### System suitability

**Test for required detectability:** Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of theophylline obtained from 20  $\mu$ L of this solution is equivalent to 15 to 25% of that of theophylline from 20  $\mu$ L of the standard solution.

**System performance:** When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.

**System repeatability:** When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of theophylline is not more than 3.0%.

**Thiamine nitrate** C<sub>12</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub>S [Same as the namesake monograph]

**Thianthol** [Same as the monograph Thianthol. Proceed as directed in the Identification (3) under Sulfur, Salicylic Acid and Thianthol Ointment: any spot other than the principal spot does not appear.]

**3-Thienylethylpenicillin sodium** C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>NaO<sub>4</sub>S<sub>2</sub>  
White to pale yellowish white powder. Very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (95).

**Optical rotation** <2.49> [ $\alpha$ ]<sub>D</sub><sup>20</sup>: +265 – +290° (0.5 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

**Water** <2.48>: Not more than 10.0% (0.2 g, volumetric titration).

**Content:** not less than 90% calculated on the anhydrous basis. Assay—Weigh accurately about 0.1 g of 3-thienylethylpenicillin sodium, dissolve in 35 mL of water, add 0.75 mL of 0.1 mol/L hydrochloric acid TS, and adjust to pH 8.5 with 0.1 mol/L sodium hydroxide TS. To this solution add 2 mL of a penicillinase solution prepared by dissolving penicillinase, equivalent to 513,000 Levy units, in 25 mL of water and neutralizing with dilute sodium hydroxide TS until a pale red color appears with 1 drop of a solution of phenolphthalein in ethanol (95) (1 in 1000) as indicator, and allow to stand at 25°C for 5 minutes. Titrate <2.50> this solution with 0.1 mol/L sodium hydroxide VS until the solution

reaches to pH 8.5 (potentiometric titration). Use the water freshly boiled and cooled.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 36.24 mg of C<sub>14</sub>H<sub>15</sub>N<sub>2</sub>NaO<sub>4</sub>S<sub>2</sub>

**Thimerosal** C<sub>9</sub>H<sub>9</sub>HgNaO<sub>2</sub>S White or yellowish crystalline powder. Freely soluble in water.

**Melting point** <2.60>: 107 – 114°C.

**Thioacetamide** C<sub>2</sub>H<sub>5</sub>NS A white crystalline powder or colorless crystals, having a characteristic odor. Freely soluble in water and in ethanol (99.5). Melting point: 112 – 115°C

**Thioacetamide-alkaline glycerin TS** To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of alkaline glycerin TS, and heat for 20 seconds in a water bath. Prepare before use.

**Thioacetamide TS** To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of a mixture of 15 mL of sodium hydroxide TS, 5 mL of water and 20 mL of 85% glycerin, and heat in a water bath for 20 seconds. Prepare before use.

**Thiodiglycol** S(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>2</sub> [ $\beta$ -Thiodiglycol for amino acid autoanalysis] Colorless or pale yellow, clear liquid.

**Specific gravity** <2.56>  $d_{20}^{20}$ : 1.180 – 1.190

**Water** <2.48>: not more than 0.7%.

**Thioglycolate medium I for sterility test** See fluid thioglycolate medium.

**Thioglycolate medium II for sterility test** See alternative thioglycolate medium.

**Thioglycolic acid** See mercapto acetic acid.

**Thionyl chloride** SOCl<sub>2</sub> A colorless or light yellow, clear liquid, having a pungent odor.

**Specific gravity** <2.56>  $d_{20}^{20}$ : about 1.65 (Method 3)

**Content:** not less than 95.0%. Assay—Weigh accurately 0.1 g of thionyl chloride in a weighing bottle, put the bottle in a glass-stoppered conical flask containing 50 mL of water cooled to about 5°C, stopper immediately, dissolve the sample thoroughly, and transfer the solution to a 200-mL beaker. Wash the conical flask and the weighing bottle in it with 30 mL of water, and combine the washings and the solution in the beaker. Add 1 drop of an aqueous solution of polyvinyl alcohol (100 g/L), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS  
= 5.949 mg of SOCl<sub>2</sub>

**Thiopental for assay** C<sub>11</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>S Dissolve 10 g of Thiopental Sodium in 300 mL of water. To this solution add slowly 50 mL of dilute hydrochloric acid with stirring. Take the produced crystals by filtration, wash with water until the filtrate indicates no reaction to chloride, and air-dry. Add diluted ethanol (99.5) (3 in 5), dissolve by heating in a water bath, allow to stand, and take the produced crystals by filtration. Air-dry the crystals in air, and dry again at 105°C for 4 hours. White, odorless crystals.

**Melting point** <2.60>: 159 – 162°C

**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of thiopental for assay in dehydrated ethanol: the solution is clear and light yellow.

(2) Related substances—Dissolve 0.05 g of thiopental for assay in 15 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase in the Purity (4) under Thiopental Sodium to make exactly 200 mL, and use this solution as the standard solution. Proceed as directed in Purity (4) under Thiopental Sodium.

**Loss on drying** <2.41>: not more than 0.20% (1 g, 105°C, 3 hours).

**Content**: not less than 99.0%. **Assay**—Weigh accurately about 0.35 g of thiopental for assay, previously dried, dissolve in 5 mL of dehydrated ethanol and 50 mL of chloroform, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 24.23 mg of  $C_{11}H_{18}N_2O_2S$

**Thiopental sodium**  $C_{11}H_{17}N_2NaO_2S$  [Same as the namesake monograph]

**Thiosemicarbazide**  $H_2NCSNHNH_2$  White crystals or crystalline powder.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3370  $cm^{-1}$ , 3180  $cm^{-1}$ , 1648  $cm^{-1}$ , 1622  $cm^{-1}$ , 1535  $cm^{-1}$ , 1288  $cm^{-1}$ , 1167  $cm^{-1}$ , 1003  $cm^{-1}$  and 803  $cm^{-1}$ .

**Thiourea**  $H_2NCSNH_2$  [K 8635, Special class]

**Thiourea TS** Dissolve 10 g of thiourea in water to make 100 mL.

**L-Threonine**  $C_4H_9NO_3$  [Same as the namesake monograph]

**Thioprocaterol 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 reflux 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 0.10 g of threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 2  $\mu L$  of the sample solution as directed under Liquid Chromatography according to the operating conditions in the Purity (3) under Procaterol Hydrochloride Hydrate. Measure each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the area percentage method: it shows the purity of not less than 95.0%. Adjust the detection sensitivity so that the peak height of threoprocaterol ob-

tained from 2  $\mu L$  of the solution prepared by diluting 5.0 mL of the sample solution with diluted methanol (1 in 2) to make 100 mL, 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 beginning after the peak of solvent.

**Thrombin** [Same as the namesake monograph]

**Thymine**  $C_5H_6N_2O_2$

**Identification**—Determine the infrared absorption spectrum of thymine, previously dried at 105°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3030  $cm^{-1}$ , 1734  $cm^{-1}$ , 1676  $cm^{-1}$ , 1446  $cm^{-1}$  and 814  $cm^{-1}$ .

**Purity** Related substances—Dissolve 50 mg of thymine in 100 mL of methanol. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Proceed with 10  $\mu L$  of the sample solution as directed in the Purity (3) under Aceglutamide Aluminum: any peak does not appear at the retention time of aceglutamide.

**Thymine for liquid chromatography**  $C_5H_6N_2O_2$  Occurs as a white powder.

**Purity**—Dissolve 10 mg of the substance to be examined in 100 mL of methanol, add the mobile phase to make exactly 250 mL, and use this solution as the sample solution. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Pipet 10  $\mu L$  each of these solutions and perform the test as directed in the Purity (3) under Zidovudine. Determine the area of each peak in the sample and standard solutions by the automatic integration method: the total area of peaks other than thymine from the sample solution is not larger than that from the standard solution. However, the time span of measurement is about 10 times the retention time of thymine, beginning after the solvent peak.

**Thymol**  $CH_3C_6H_3(OH)CH(CH_3)_2$  [Same as the namesake monograph]

**Thymol blue**  $C_{27}H_{30}O_5S$  [K 8643, Special class]

**Thymol blue-N,N-dimethylformamide TS** Dissolve 0.1 g of thymol blue in 100 mL of N,N-dimethylformamide.

**Thymol blue-dioxane TS** Dissolve 0.05 g of thymol blue in 100 mL of 1,4-dioxane, and filter if necessary. Prepare before use.

**Thymol blue TS** Dissolve 0.1 g of thymol blue in 100 mL of ethanol (95), and filter if necessary.

**Thymol blue TS, dilute** Dissolve 0.05 g of thymol blue in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

**Thymol for assay** [Same as the monograph Thymol. It contains not less than 99.0% of thymol ( $C_{10}H_{14}O$ ).]

**Thymol for spraying test solution**  $C_{10}H_{14}O$  White crystals or crystalline powder, having an aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification**—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorp-

tion at the wave numbers of about 2960  $\text{cm}^{-1}$ , 1420  $\text{cm}^{-1}$ , 1290  $\text{cm}^{-1}$ , 1090  $\text{cm}^{-1}$  and 810  $\text{cm}^{-1}$ .

*Melting point* <2.60>: 49 – 52°C

*Purity* Otherphenols—Shake vigorously 1.0 g of the substance to be examined with 20 mL of warm water for 1 minute, and filter. To 5 mL of the filtrate add 1 drop of a solution of iron (III) chloride hexahydrate (27 in 100): the solution reveals a green but not a blue to purple color.

**Thymolphthalein**  $\text{C}_{28}\text{H}_{30}\text{O}_4$  [K 8642, Special class]

**Thymolphthalein TS** Dissolve 0.1 g of thymolphthalein in 100 mL of ethanol (95), and filter if necessary.

**Thymol-sulfuric acid-methanol TS for spraying** Dissolve 1.5 g of thymol for spraying test solution in 100 mL of methanol, and add 5.7 mL of sulfuric acid.

**Tiamide hydrochloride for assay**  $\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}\cdot\text{HCl}$  [Same as the monograph Tiamide Hydrochloride. When dried, it contains not less than 99.0% of tiamide hydrochloride ( $\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}\cdot\text{HCl}$ ).]

**Tiapride hydrochloride for assay**  $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_4\text{S}\cdot\text{HCl}$  [Same as the monograph Tiapride Hydrochloride]

**Tin** Sn [K 8580, Special class]

**Tin (II) chloride dihydrate**  $\text{SnCl}_2\cdot 2\text{H}_2\text{O}$  [K 8136, Special class]

**Tin (II) chloride-hydrochloric acid TS** To 20 g of tin add 85 mL of hydrochloric acid, heat until hydrogen gas no longer are evolved, and allow to cool. Mix 1 volume of this solution and 10 volume of dilute hydrochloric acid. Prepare before use.

**Tin (II) chloride-sulfuric acid TS** Dissolve 10 g of tin (II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

**Tin (II) chloride TS** Dissolve 1.5 g of Tin (II) chloride dihydrate in 10 mL of water containing a small amount of hydrochloric acid. Preserve in 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 500 mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.

**Tipepidine hibenzate for assay**  $\text{C}_{15}\text{H}_{17}\text{NS}_2\cdot\text{C}_{14}\text{H}_{10}\text{O}_4$  [Same as the monograph Tipepidine Hibenzate. When dried, it contains not less than 99.0% of  $\text{C}_{15}\text{H}_{17}\text{NS}_2\cdot\text{C}_{14}\text{H}_{10}\text{O}_4$ .]

**Titanium dioxide** See titanium (IV) oxide.

**Titanium dioxide TS** See titanium (IV) oxide TS.

**Titanium (III) chloride (20)**  $\text{TiCl}_3$  [K 8401, Titanium (III) chloride solution, Special class] Store in light-resistant, glass-stoppered containers.

**Titanium (III) chloride-sulfuric acid TS** Mix carefully 20 mL of titanium (III) chloride TS and 13 mL of sulfuric acid, add carefully hydrogen peroxide (30) in small portions until a yellow color develops, and heat until white fumes evolve. After cooling, add water, heat again in the same manner, repeat this procedure until the solution is colorless, and add water to make 100 mL.

**Titanium (III) chloride TS** To titanium (III) chloride (20)

add dilute hydrochloric acid to obtain a solution containing 15 g/dL of titanium (III) chloride ( $\text{TiCl}_3$ ). Prepare before use.

*Content*: 14.0 – 16.0 g/dL. *Assay*—To exactly 2 mL of titanium (III) chloride add 200 mL of water and 5 mL of a hydrochloric acid solution (2 in 3), and titrate <2.50> with 0.1 mol/L ferric ammonium sulfate VS under carbon dioxide until a slight red color develops in the solution (indicator: 5 mL of ammonium thiocyanate TS).

Each mL of 0.1 mol/L ferric ammonium sulfate VS = 15.42 mg of  $\text{TiCl}_3$

**Titanium (IV) oxide**  $\text{TiO}_2$  [K 8703, Special class]

**Titanium (IV) oxide TS** To 100 mL of sulfuric acid add 0.1 g of titanium (IV) oxide, and dissolve by gradually heating on a flame 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) chloride TS.

**Titanium yellow**  $\text{C}_{28}\text{H}_{19}\text{N}_5\text{Na}_2\text{O}_6\text{S}_4$  A dark yellow to dark yellow-brown powder or masses.

*Identification*—Determine the infrared absorption spectrum of titanium yellow, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1603  $\text{cm}^{-1}$ , 1467  $\text{cm}^{-1}$ , 1394  $\text{cm}^{-1}$ , 1306  $\text{cm}^{-1}$ , 1040  $\text{cm}^{-1}$ , 988  $\text{cm}^{-1}$ , 820  $\text{cm}^{-1}$  and 644  $\text{cm}^{-1}$ .

Preserve in a light-resistant tight container.

**Tocopherol**  $\text{C}_{29}\text{H}_{50}\text{O}_2$  [Same as the namesake monograph]

**Tocopherol acetate**  $\text{C}_{31}\text{H}_{52}\text{O}_3$  [Same as the namesake monograph]

**Tocopherol calcium succinate**  $\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$  [Same as the namesake monograph]

**Tocopherol succinate**  $\text{C}_{33}\text{H}_{54}\text{O}_5$  Wet 0.5 g of tocopherol calcium succinate with 5 mL of acetic acid (100), add 10 mL of toluene, and warm at 70°C for 30 minutes with occasional shaking. After cooling, add 30 mL of water, shake thoroughly, and allow to stand. Remove the water layer, wash the toluene layer with several 30-mL portions 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* <2.24>  $E_{1\text{cm}}^{1\%}$  (286 nm): 38.0 – 42.0 (10 mg, chloroform, 100 mL).

**Tolbutamide**  $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$  [Same as the namesake monograph]

**Toluene**  $\text{C}_6\text{H}_5\text{CH}_3$  [K 8680, Special class]

**o-Toluene sulfonamide**  $\text{C}_7\text{H}_9\text{NO}_2\text{S}$  Colorless crystals or white crystalline powder. Soluble in ethanol (95), and sparingly soluble in water.

*Melting point* <2.60>: 157 – 160°C

**Purity** *p*-Toluene sulfonamide—Use a solution of *o*-toluene sulfonamide in ethyl acetate (1 in 5000) as the sample solution. Perform the test with 10  $\mu$ L of the sample solution as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Saccharin Sodium Hydrate: 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  $\mu$ L of the sample 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 beginning after the solvent peak.

**Water** <2.48>: not more than 0.5% (4 g, use 25 mL of methanol for Karl Fischer method and 5 mL of pyridine for Karl Fischer method).

**Content**: not less than 98.5%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 0.025 g of *o*-toluene sulfonamide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS  
= 1.712 mg of C<sub>7</sub>H<sub>9</sub>NO<sub>2</sub>S

***p*-Toluene sulfonamide** CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NH<sub>2</sub> White, crystals or crystalline powder. Melting point: about 137°C

**Purity** Related substances—Dissolve 30 mg of *p*-toluene sulfonamide in acetone to make exactly 200 mL. Proceed with 10  $\mu$ L of this solution as directed in the Purity (3) under Tolazamide: any spot other than the principal spot at the *R<sub>f</sub>* value of about 0.6 does not appear.

***p*-Toluene sulfonic acid** See *p*-toluenesulfonic acid monohydrate.

***p*-Toluenesulfonic acid monohydrate**  
CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>H·H<sub>2</sub>O [K 8681, Special class]

***o*-Toluic acid** C<sub>8</sub>H<sub>8</sub>O<sub>2</sub> White, crystals or crystalline powder.

**Melting point** <2.60>: 102 – 105°C

**Content**: not less than 98.0%.

**Toluidine blue** See toluidine blue O

**Toluidine blue O** C<sub>15</sub>H<sub>16</sub>ClN<sub>3</sub>S Dark green powder, soluble in water, and slightly soluble in ethanol (95).

**Identification**—

- (1) A solution (1 in 100) shows a blue to purple color.
- (2) A solution in ethanol (95) (1 in 200) shows a blue color.
- (3) A solution shows a maximum absorption at around 630 nm.

**Triamcinolone acetonide** C<sub>24</sub>H<sub>31</sub>FO<sub>6</sub> [Same as the namesake monograph]

**Trichloroacetic acid** CCl<sub>3</sub>COOH [K 8667, Special class]

**Trichloroacetic acid-gelatin-tris buffer solution** To 1 volume of a solution of trichloroacetic acid (1 in 5) add 6 volume of gelatin-tris buffer solution, pH 8.0 and 5 volume of water.

**Trichloroacetic acid TS** Dissolve 1.80 g of trichloroacetic acid, 2.99 g of sodium acetate trihydrate and 1.98 g of acetic acid (31) in water to make 100 mL.

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

**Trichlorofluoromethane** CCl<sub>3</sub>F A colorless liquid or gas.

**Specific gravity** <2.56> *d*<sub>4</sub><sup>17.2</sup>: 1.494

**Boiling point** <2.57>: 23.7°C

**1,1,2-Trichloro-1,2,2-trifluoroethane** CFCl<sub>2</sub>.CF<sub>2</sub>Cl Colorless volatile liquid. Miscible with acetone and with diethyl ether, and not with water.

**Purity** Related substances—Perform the test with 0.1  $\mu$ L of 1,1,2-trichloro-1,2,2-trifluoroethane as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Halothane: any peak other than the peak of 1,1,2-trichloro-1,2,2-trifluoroethane does not appear.

**Tricine** C<sub>6</sub>H<sub>13</sub>NO<sub>5</sub> White crystalline powder. Melting point: 182 to 184°C (with decomposition).

**Triethanolamine** See 2,2',2''-nitrilotriethanol.

**Triethylamine** (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N Clear colorless liquid, having a strong amines odor. Miscible with methanol, with ethanol (95) and with diethyl ether.

**Specific gravity** <2.56> *d*<sub>4</sub><sup>20</sup>: 0.722 – 0.730

**Boiling point** <2.57>: 89 – 90°C

**Triethylamine buffer solution, pH 3.2** To 4 mL of triethylamine add 2000 mL of water, and adjust the pH to 3.2 with phosphoric acid.

**Triethylamine-phosphate buffer solution, pH 5.0** To 1.0 mL of triethylamine add 900 mL of water, adjust the pH to 5.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

**1% Triethylamine-phosphate buffer solution, pH 3.0** Dissolve 10 g of triethylamine in 950 mL of water, adjust the pH to 3.0 with phosphoric acid, and make exactly 1000 mL.

**Trifluoroacetic acid** CF<sub>3</sub>COOH Colorless, clear liquid, having a pungent odor. Miscible well with water.

**Specific gravity** <2.56> *d*<sub>20</sub><sup>20</sup>: 1.535

**Boiling point** <2.57>: 72 – 73°C

**Trifluoroacetic acid for nuclear magnetic resonance spectroscopy** CF<sub>3</sub>COOH Prepared for nuclear magnetic resonance spectroscopy.

**Trifluoroacetic acid TS** To 1 mL of trifluoroacetic acid add water to make 1000 mL.

**Trifluoroacetic anhydride for gas chromatography** (CF<sub>3</sub>CO)<sub>2</sub>O Colorless, clear liquid, having a pungent odor.

**Boiling point** <2.57>: 40 – 45°C

**Trimetazidine hydrochloride for assay** C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>·2HCl [Same as the monograph Trimetazidine Hydrochloride. It contains not less than 99.0% of trimetazidine hydrochloride (C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>·2HCl), calculated on the anhydrous basis.]

**Trimethylsilyl imidazole** C<sub>6</sub>H<sub>12</sub>N<sub>2</sub>Si Clear, colorless to pale yellow liquid.

**Refractive index** <2.45> *n*<sub>D</sub><sup>20</sup>: 1.4744 – 1.4764

**2,4,6-trinitrobenzenesulfonic acid** See 2,4,6-trinitroben-

zenesulfonic acid dihydrate.

**2,4,6-Trinitrobenzenesulfonic acid dihydrate**

$C_6H_2(NO_2)_3SO_3H \cdot 2H_2O$  Pale yellow to light yellow powder.

*Water* <2.48>: 11 – 15% (0.1 g, 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 50 mL of a mixture of water and ethanol (99.5) (1:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 29.32 mg of  $C_6H_2(NO_2)_3SO_3H$

**2,4,6-Trinitrophenol**  $HOC_6H_2(NO_2)_3$  Light yellow to yellow, moist crystals. It is added 15 to 25% of water for the sake of safety, because it might explode by heating, mechanical shocking and friction when it is dried.

*Identification*—To 0.1 g add 10 mL of water, dissolve by warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

*Content*: not less than 99.5%. Assay—Weigh accurately about 0.25 g, previously dried in a desiccator (silica gel) for 24 hours, dissolve in 50 mL of water by warming, and titrate <2.50> 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.

Each mL of 0.1 mol/L sodium hydroxide VS  
= 22.91 mg of  $HOC_6H_2(NO_2)_3$

**2,4,6-Trinitrophenol-ethanol TS** Dissolve 1.8 g of 2,4,6-trinitrophenol in 50 mL of diluted ethanol (99.5) (9 in 10) and 30 mL of water, and add water to make 100 mL.

**2,4,6-Trinitrophenol TS** Dissolve 1 g of 2,4,6-trinitrophenol in 100 mL of hot water, cool, and filter if necessary.

**2,4,6-Trinitrophenol TS, alkaline** Mix 20 mL of 2,4,6-trinitrophenol TS with 10 mL of a solution of sodium hydroxide (1 in 20), and add water to make 100 mL. Use within 2 days.

**Triphenylchloromethane**  $(C_6H_5)_3CCl$  White to grayish or yellowish white, crystals or crystalline powder.

*Melting point* <2.60>: 107 – 115°C

**Triphenylmethanol for thin-layer chromatography**

$C_{19}H_{15}OH$  Occurs as a white powder.

*Purity*—Dissolve 0.1 g of triphenylmethanol for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity (2) under Zidovudine: spots other than the principal spot with an  $R_f$  value of about 0.73 are not observed.

**Triphenyltetrazolium chloride** See 2,3,5-triphenyl-2H-tetrazolium chloride.

**Triphenyltetrazolium chloride TS** See 2,3,5-triphenyl-2H-tetrazolium chloride TS.

**2,3,5-Triphenyl-2H-tetrazolium chloride**  $C_{19}H_{15}ClN_4$   
[K 8214, Special class]

**2,3,5-Triphenyl-2H-tetrazolium chloride TS** Dissolve 0.25 g of 2,3,5-triphenyl-2H-tetrazolium chloride in ethanol (99.5) to make 100 mL. Prepare before use.

**2,3,5-Triphenyl-2H-tetrazolium chloride-methanol TS for spraying** Solution A: A solution of 2,3,5-triphenyl-2H-tetrazolium chloride in methanol (1 in 25). Solution B: A solution of sodium hydroxide in methanol (1 in 125). Mix an equal volume of the solution A and solution B just before use.

**Tripotassium citrate monohydrate**  $C_6H_5K_3O_7 \cdot H_2O$   
White crystals or crystalline powder. Very soluble in water, and practically insoluble in ethanol (95).

*Content*: 99.0% or more Assay—Accurately weigh about 0.2 g of tripotassium citrate monohydrate, add 50 mL of acetic acid for nonaqueous titration, dissolve by warming on a water bath, cool, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Correct by conducting a blank test using the same method.

Each mL of 0.1 mol/L perchloric acid VS  
= 32.44 mg of  $C_6H_5K_3O_7 \cdot H_2O$

**Tris buffer solution for bacterial endotoxins test** Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water for bacterial endotoxins test, add 100 mL of 0.1 mol/L hydrochloric acid TS and water for bacterial endotoxins test to make 1000 mL, and sterilize by heating in an autoclave at 121°C for 90 minutes.

**Tris-acetic acid buffer solution, pH 6.5** Dissolve 13.57 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 6.73 g of acetic acid (100) in water to make 1000 mL.

**Tris buffer solution, pH 7.0** Dissolve 24.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid TS.

**Tris buffer solution, pH 8.2** Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.5 g of polysorbate 20 in 800 mL of water, adjust to pH 8.2 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris buffer solution, pH 8.4** Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 10.2 g of sodium chloride in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**Tris buffer solution, pH 9.5** Dissolve 36.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 9.5 by adding 1 mol/L hydrochloric acid TS.

**1.5 mol/L Tris buffer solution, pH 8.8** Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 75 mL of water, add 5 mol/L hydrochloric acid TS to adjust the pH to 8.8, and then add water to make 100 mL. Filter if necessary.

**0.5 mol/L Tris buffer solution, pH 6.8** Dissolve 6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 50 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 6.8, and then add water to make 100 mL. Filter if necessary.

**0.1 mol/L Tris buffer solution, pH 8.0** Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 100 mL of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

**0.05 mol/L Tris buffer solution, pH 7.0** Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in about 750 mL of water, adjust to pH 7.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

**0.05 mol/L Tris buffer solution, pH 8.6** Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 950 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 8.6, and then add water to make 1000 mL.

**Tris(4-*t*-butylphenyl)phosphate**  $[(\text{CH}_3)_3\text{CC}_6\text{H}_4\text{O}]_3\text{PO}$   
White crystals or crystalline powder.  
*Melting point* <2.60>: 100 – 104°C

**0.2 mol/L Tris-hydrochloride buffer solution, pH 7.4**  
Dissolve 6.61 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 0.97 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 250 mL.

**0.05 mol/L Tris-hydrochloride buffer solution, pH 7.5**  
Dissolve 6.35 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 1.18 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL.

**Trishydroxymethylaminomethane** See 2-amino-2-hydroxymethyl-1,3-propanediol.

**Trisodium citrate dihydrate** See sodium citrate hydrate.

**0.1 mol/L Trisodium citrate TS** Dissolve 29.4 g of trisodium citrate dihydrate in water to make 1000 mL.

**Trisodium ferrous pentacyanoamine TS** See iron (II) trisodium pentacyanoamine TS.

**Trisodium phosphate dodecahydrate**  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$   
[K 9012, Special class]

**Trypsin for liquid chromatography** An enzyme obtained from the bovine pancreas. This one part digests 250 parts of casein in the following reaction system.

**Casein solution**—To 0.1 g of milk casein add 30 mL of water, disperse the casein well, add 1.0 mL of diluted sodium hydroxide TS (1 in 10) to dissolve, and add water to make 50 mL. Prepare before use.

**Sample solution**—Dissolve 0.01 g of trypsin for liquid chromatography in 500 mL of water.

**Procedure**—To 5 mL of the casein solution add 2 mL of the sample solution and 3 mL of water, mix, then allow to stand at 40°C for 1 hour, and add 3 drops of a mixture of ethanol (95), water and acetic acid (100) (10:9:1): no precipitate appears.

**Trypsin inhibitor** Produced by purifying soybean. Each mg of trypsin inhibitor inhibits 10,000 to 30,000 BAEE Units of trypsin. One BAEE Unit means a trypsin activity to indicate an absorbance difference of 0.001 at 253 nm when 3.2 mL of the solution is reacted at 25°C and pH 7.6, using *N*- $\alpha$ -benzoyl-L-arginine ethyl ester as substrate.

**Trypsin inhibitor TS** Dissolve 5 mg of trypsin inhibitor in 0.05 mol/L phosphate buffer solution, pH 7.0 to make 10 mL.

**Trypsin TS for test of elcatonin** Dissolve 5 mg of trypsin for liquid chromatography in 20 mL of a solution of ammonium hydrogen carbonate (1 in 100). Prepare before use.

**Trypsin TS for test of ulinastatin** Dissolve crystalline trypsin for ulinastatin assay in ice-cooled 1 mmol/L hydro-

chloric acid TS containing 1 mmol/L calcium chloride dihydrate so that each mL of the solution contains 180  $\mu\text{g}$  of trypsin. Prepare before use, and preserve in an ice-cooled water bath.

**L-Tryptophan**  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$  [Same as the namesake monograph]

**Turpentine oil** [Same as the namesake monograph]

**L-Tyrosine**  $\text{C}_9\text{H}_{11}\text{NO}_3$  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* <2.49>  $[\alpha]_D^{20}$ : –10.5 – –12.5° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

*Loss on drying* <2.41>: 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 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS  
= 18.12 mg of  $\text{C}_9\text{H}_{11}\text{NO}_3$

**Ubenimex for assay**  $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4$  [Same as the monograph Ubenimex. When dried, it contains not less than 99.0% of ubenimex ( $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4$ ).]

**Ubiquinone-9** Yellow to orange, crystalline powder. Odorless and no taste.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (275 nm): 163 – 190 (ethanol (99.5))

*Melting point* <2.60>: about 44°C

**Uracil**  $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$  Needle crystals. Freely soluble in hot water, and slightly soluble in cold water.

*Melting point* <2.60>: 335°C

**Urea**  $\text{H}_2\text{NCONH}_2$  [K 8731, Special class]

**Urethane** See ethyl carbamate.

**Ursodeoxycholic acid**  $\text{C}_{24}\text{H}_{40}\text{O}_4$  [Same as the namesake monograph]

**Ursodeoxycholic acid for assay**  $\text{C}_{24}\text{H}_{40}\text{O}_4$  [Same as the monograph Ursodeoxycholic Acid. However, when dried, it contains not less than 99.0% of ursodeoxycholic acid ( $\text{C}_{24}\text{H}_{40}\text{O}_4$ ) meeting the following additional specifications.]

*Purity* Related substances—Dissolve 0.15 g of ursodeoxycholic acid for assay in 5 mL of methanol for liquid chromatography, and use this solution as the sample solution. Pipet 2 mL of this solution and add methanol for liquid chromatography to make exactly 50 mL. Pipet 2.5 mL of this solution, add methanol for liquid chromatography to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5  $\mu\text{L}$  each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.5 with respect to ursodeoxycholic acid, obtained from the sample solution is not larger

than the peak area of ursodeoxycholic acid from the standard solution, and the area of the peak, having the relative retention time of about 5.5, obtained from the sample solution is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution. Furthermore, the total area of the peaks other than the peak of ursodeoxycholic acid and other than the peaks mentioned above is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution.

#### Operating conditions

**Detector:** An ultraviolet absorption photometer (wavelength: 210 nm).

**Column:** A stainless steel column 3 mm in inside diameter and 7.5 cm in length, packed with octylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 40°C.

**Mobile phase:** A mixture of methanol for liquid chromatography, diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (96:69:35).

**Flow rate:** Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 2.3 minutes.

**Time span of measurement:** About 7 times as long as the retention time of ursodeoxycholic acid.

#### System suitability

**Test for required detectability:** Pipet 2 mL of the standard solution, and add methanol for liquid chromatography to make exactly 20 mL. Confirm that the peak area of ursodeoxycholic acid obtained from 5  $\mu$ L of this solution is equivalent to 8 to 12% of that of ursodeoxycholic acid from 5  $\mu$ L of the standard solution.

**System performance:** To 30 mg of chenodeoxycholic acid for thin-layer chromatography and 30 mg of lithocholic acid for thin-layer chromatography, add 1 mL of the sample solution, dissolve in methanol for liquid chromatography to make 50 mL. When the procedure is run with 5  $\mu$ L of this solution under the above operating conditions, ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid are eluted in this order with the resolution between these peaks being not less than 7, respectively.

**System repeatability:** When the test is repeated 6 times with 5  $\mu$ L of the standard solution under the above conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0%.

***n*-Valerianic acid**  $\text{CH}_3(\text{CH}_2)_3\text{COOH}$  Clear, colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and with diethyl ether, and soluble in water.

*Specific gravity* <2.56>  $d_4^{20}$ : 0.936 – 0.942

*Distilling range* <2.57>: 186 – 188°C, not less than 98 vol%.

**L-Valine**  $\text{C}_5\text{H}_{11}\text{NO}_2$  [Same as the namesake monograph]

**L-Valine for assay**  $\text{C}_5\text{H}_{11}\text{NO}_2$  [Same as the monograph L-Valine. When dried, it contains not less than 99.0% of L-valine ( $\text{C}_5\text{H}_{11}\text{NO}_2$ ).]

**H-D-Valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride**  $\text{C}_{23}\text{H}_{38}\text{N}_8\text{O}_5 \cdot 2\text{HCl}$  White to pale yellow, powder or masses. Sparingly soluble in water.

*Absorbance* <2.24>  $E_{1\text{cm}}^{1\%}$  (316 nm): 214 – 236 (0.01 g, water, 500 mL).

**Vanadium pentoxide** See vanadium (V) oxide.

**Vanadium pentoxide TS** See vanadium (V) oxide TS.

**Vanadium pentoxide TS, dilute** See vanadium (V) oxide TS, dilute.

**Vanadium (V) oxide**  $\text{V}_2\text{O}_5$  Orangish yellow to yellow-brown powder.

*Identification*—Dissolve 0.3 g in 10 mL of ammonia TS and 15 mL of water. To 2 mL of this solution add 20 mL of water, mix, and add gently 1 mL 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 10 mL of vanadium (V) oxide TS with water to make 100 mL. Prepare before use.

**Vanillin**  $\text{C}_6\text{H}_3\text{CHO}(\text{OCH}_3)(\text{OH})$  A white to light yellow crystalline powder, having a characteristic odor.

*Melting point* <2.60>: 80.5 – 83.5°C.

Preserve in a light-resistant tight container.

**Vanillin-hydrochloric acid TS** Dissolve 5 mg of vanillin in 0.5 mL of ethanol (95), and to this solution add 0.5 mL of water and 3 mL of hydrochloric acid. Prepare before use.

**Vanillin-sulfuric acid-ethanol TS** Dissolve 3 g of vanillin in ethanol (99.5) to make 100 mL, and add 0.5 mL of sulfuric acid.

**Vanillin-sulfuric acid-ethanol TS for spraying** Dissolve 3 g of vanillin in 30 mL of ethanol (99.5), and add 100 mL of dilute sulfuric acid.

**Vanillin-sulfuric acid TS** Add cautiously 75 mL of sulfuric acid to 25 mL of ice-cold ethanol (95). After cooling, add 1 g of vanillin to dissolve. Prepare before use.

**Vasopressin**  $\text{C}_{46}\text{H}_{65}\text{N}_{15}\text{O}_{12}\text{S}_2$  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.

**Verapamil hydrochloride for assay**  $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$  [Same as the monograph Verapamil Hydrochloride. When dried, it contains not less than 99.0% of verapamil hydrochloride ( $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$ ).]

**Vinblastine sulfate**  $\text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9 \cdot \text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Vincristine sulfate**  $\text{C}_{46}\text{H}_{56}\text{N}_4\text{O}_{10} \cdot \text{H}_2\text{SO}_4$  [Same as the namesake monograph]

**Vinyl acetate**  $\text{C}_4\text{H}_6\text{O}_2$  Clear, colorless liquid.

*Specific gravity* <2.56>: 0.932 – 0.936

*Water* <2.48>: not more than 0.2%

**Vinyl chloride**  $\text{C}_2\text{H}_3\text{Cl}$  Colorless gas.

*Boiling point* <2.57>: –14°C

*Melting point* <2.60>: –160°C

**2-Vinylpyridine**  $C_7H_7N$  A clear, colorless or dark brown liquid.

*Refractive index*  $\langle 2.45 \rangle$   $n_D^{20}$ : 1.546 – 1.552

*Specific gravity*  $\langle 2.56 \rangle$   $d_{20}^{20}$ : 0.975 – 0.982

**1-Vinyl-2-pyrrolidone**  $C_6H_9NO$  Clear liquid.

*Purity*—Perform the test with 0.5  $\mu$ L of 1-vinyl-2-pyrrolidone as directed under Gas Chromatography  $\langle 2.02 \rangle$  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- $\mu$ m 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, and hold constant to the temperature for 20 minutes.

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  $\mu$ L of 1-vinyl-2-pyrrolidone is about 70% of the full scale.

Time span of measurement: About twice as long as the retention time of 1-vinyl-2-pyrrolidone beginning after the solvent peak.

*Water*  $\langle 2.48 \rangle$ —Take 50 mL of methanol for Karl Fischer method and 10 mL 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%.

**V8 protease** A protease obtained from *Staphylococcus aureus* strain. When an amount of the enzyme hydrolyzes 1  $\mu$ mol of *N*-*t*-butoxycarbonyl-L-glutamic acid- $\alpha$ -phenyl ester in 1 minute at pH 7.8 and 37°C is defined as 1 unit, it contains 500 – 1000 units per mg.

**V8 protease TS** Dissolve V8 protease in water to make a solution of 1 mg/mL. Keep at a cold place and use within 6 days after preparation.

**Voglibose for assay**  $C_{10}H_{21}NO_7$  [Same as the monograph Voglibose]

**Warfarin potassium for assay** [Same as the monograph Warfarin Potassium. When dried, it contains not less than 99.0% of warfarin potassium ( $C_{19}H_{15}KO_4$ ).]

**25% Water containing benzoyl peroxide** See Benzoyl peroxide, 25% water containing.

**Water for ammonium limit test** To 1500 mL of water add cautiously 4.5 mL of sulfuric acid, distil using a hard glass distiller, discard the sufficient volume of first distillate, and use the remaining distillate (ammonium-free water) as the water for ammonium limit test.

*Purity*—Mix 40 mL of water for ammonium limit test with 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS.

Add 4.0 mL of sodium hypochlorite-sodium hydroxide TS, mix, and allow to stand for 60 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry  $\langle 2.24 \rangle$ , using water as the blank: the absorbance at 640 nm is not more than 0.010.

**Water for bacterial endotoxins test** [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers, or the water produced by other procedures that shows no reaction with the lysate reagent employed, at the detection limit of the reagent, and is suitable for bacterial endotoxins test.]

**Water for injection** [Use the water prescribed by the monographs of Water for Injection or Sterile Water for Injection in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

**Water, sterile purified** [Use the water prescribed by the monograph of Sterile Purified Water in Containers. It is not necessary to check the conformity to all the specification items of the monograph, if it is confirmed that the water to be used is suitable for the purpose of relevant test.]

**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 1000 mL. Preserve in light-resistant, glass containers.

**Wogonin for thin-layer chromatography**  $C_{16}H_{12}O_5$  Yellow crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 204 – 208°C

*Identification*—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry  $\langle 2.24 \rangle$ : it exhibits maxima between 207 nm and 211 nm, and between 273 nm and 277 nm.

*Purity* Related substances—Dissolve 1 mg in 1 mL of methanol, and perform the test with 10  $\mu$ L of this solution as directed in the Identification (3) under Saireito Extract: no spot other than the principal spot (*Rf* value is about 0.4) appears.

**Xanthene**  $C_{13}H_{10}O$  White to light yellow crystals or crystalline powder, having a slight, characteristic odor.

*Melting point*  $\langle 2.60 \rangle$ : 98 – 102°C

*Water*  $\langle 2.48 \rangle$ : not more than 0.5% (0.15 g).

**Xanthene-9-carboxylic acid**  $C_{14}H_{10}O_3$  Dissolve 0.25 g of propantheline bromide in 5 mL of water and 10 mL of sodium hydroxide TS, heat the mixture to boiling, then continue to heat for 2 minutes. Cool to 60°C, add 5 mL of dilute sulfuric acid, cool, filter the precipitate, and wash thoroughly with water. Recrystallize the residue from dilute ethanol, and dry for 3 hours in a desiccator (in vacuum, silica gel).

*Melting point*  $\langle 2.60 \rangle$ : 217 – 222°C

**Xanthone**  $C_{13}H_8O_2$  Light yellow powder. Freely soluble in chloroform, and slightly soluble in hot water and in diethyl ether.

*Melting point*  $\langle 2.60 \rangle$ : 174 – 176°C

*Purity* Related substances—Dissolve 0.050 g of xanthone in chloroform to make exactly 10 mL. Perform the test with 5  $\mu$ L of this solution as directed in the Purity under Propan-

theline Bromide: any spot other than the principal spot at the Rf value of about 0.7 does not appear.

**Xanthhydrol**  $C_{13}H_{10}O_2$  White to pale yellow powder. Dissolves in ethanol (95), in diethyl ether, in chloroform, and in acetic acid (100), and is practically insoluble in water.

*Melting point* <2.60>: 121 – 124°C

*Residue on ignition* <2.44>: not more than 2.0% (0.5 g).

**Xylene**  $C_6H_4(CH_3)_2$  [K 8271, First class]

**o-Xylene**  $C_6H_4(CH_3)_2$  Colorless, clear liquid.

*Refractive index* <2.45>  $n_D^{20}$ : 1.501 – 1.506

*Specific gravity* <2.56>  $d_4^{20}$ : 0.875 – 0.885

*Distilling range* <2.57>: 143 – 146°C, not less than 95 vol%.

**Xylene cyanol FF**  $C_{25}H_{27}N_2NaO_7S_2$  [K 8272, Special class]

**Xylenol orange**  $C_{31}H_{30}N_2Na_2O_{13}S$  [K 9563, Special class]

**Xylenol orange TS** Dissolve 0.1 g of xylenol orange in water to make 100 mL.

**Xylitol**  $C_5H_{12}O_5$  [Same as the namesake monograph]

**Xylose** See D-xylose.

**D-Xylose**  $C_5H_{10}O_5$  [Same as the monograph D-Xylose of the Japanese 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 <1.03> (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* <2.41>: not more than 5% (105°C, constant mass).

*Residue on ignition* <2.44>: not more than 15% (0.5 g).

*Nitrogen content* <1.08>: 7.2 – 9.5% (105°C, constant mass, after drying).

**Yellow beeswax** [Same as the namesake monograph]

**Zaltoprofen**  $C_{17}H_{14}O_3S$  [Same as the namesake monograph]

**Zaltoprofen for assay**  $C_{17}H_{14}O_3S$  [Same as the monograph Zaltoprofen. When dried, it contains not less than 99.5% of zaltoprofen ( $C_{17}H_{14}O_3S$ )].

**Zanthoxylum Fruit** [Same as the namesake monograph]

**Zinc** Zn [K 8012, Special class]

**Zinc acetate** See zinc acetate dihydrate.

**0.25 mol/L Zinc acetate buffer solution, pH 6.4** Dissolve 54.9 g of zinc acetate dihydrate in 150 mL of acetic acid (100) and 600 mL of water, add 150 mL of ammonia water (28), gently mix, and allow to cool to a room temperature. Adjust to pH 6.4 with ammonia water (28), and add

water to make 1000 mL.

**Zinc acetate dihydrate**  $Zn(CH_3COO)_2 \cdot 2H_2O$  [K 8356, Special class]

**Zinc, arsenic-free** See zinc for arsenic analysis.

**Zinc chloride**  $ZnCl_2$  [K 8111, Special class]

**Zinc chloride TS** Dissolve 10 g of zinc chloride and 10 g of potassium hydrogen phthalate in 900 mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

**0.04 mol/L Zinc chloride TS** Dissolve 5.452 g of zinc chloride in water to make 1000 mL.

**Zinc diethyldithiocarbamate** See Test Methods for Plastic Containers <7.02>.

**Zinc dibutyldithiocarbamate** See Test Methods for Plastic Containers <7.02>.

**Zinc disodium ethylenediamine tetraacetate** See zinc disodium ethylenediamine tetraacetate tetrahydrate.

**Zinc disodium ethylenediamine tetraacetate tetrahydrate**  $C_{10}H_{12}N_2Na_2O_8Zn \cdot 4H_2O$  White powder. The pH of a solution of zinc disodium ethylenediamine tetraacetate (1 in 100) is between 6.0 and 9.0.

*Purity* Clarity and color of solution—Dissolve 0.10 g of zinc disodium ethylenediamine tetraacetate tetrahydrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

*Content*: not less than 98.0%. Assay—Dissolve about 0.5 g of zinc disodium ethylenediamine tetraacetate tetrahydrate, accurately weighed, in water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH to about 2 with 80 mL of water and dilute nitric acid, and titrate <2.50> 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).

Each mL of 0.01 mol/L bismuth nitrate VS  
= 4.716 mg of  $C_{10}H_{12}N_2Na_2O_8Zn \cdot 4H_2O$

**Zinc dust** See zinc powder.

**Zinc for arsenic analysis** Zn [K 8012] Use granules of about 800  $\mu m$ .

**Zinc iodide-starch TS** To 100 mL of boiling water add a solution of 0.75 g of potassium iodide in 5 mL of water, a solution of 2 g of zinc chloride in 10 mL of water and a smooth suspension of 5 g of starch in 30 mL of water, with stirring. Continue to boil for 2 minutes, then cool.

*Sensitivity*—Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitrite VS, 500 mL of water and 10 mL 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**  $C_{20}H_{15}N_4NaO_6S$  A dark red to purple powder.  
*Identification*—Determine the infrared absorption spectrum of zincon, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method as directed under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1604  $cm^{-1}$ , 1494  $cm^{-1}$ , 1294  $cm^{-1}$ , 1194  $cm^{-1}$ , 1110  $cm^{-1}$ , 1046  $cm^{-1}$  and 764  $cm^{-1}$ .

Preserve in a light-resistant tight container.

**Zincon TS** Dissolve 0.1 g of zincon in 2 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL.

**Zinc powder** Zn [K 8013, for nitrogen oxides analysis or arsenic analysis]

**Zinc (standard reagent)** Zn [K 8005, Standard reagent for volumetric analysis]

**Zinc sulfate** See zinc sulfate heptahydrate.

**Zinc sulfate for volumetric analysis** See zinc sulfate heptahydrate.

**Zinc sulfate heptahydrate**  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  [K 8953, Special class]

**Zinc sulfate TS** Dissolve 10 g of zinc sulfate heptahydrate in water to make 100 mL.

**Zirconyl-alizarin red S TS** Dissolve 0.2 g of zirconyl nitrate in 5 mL of dilute hydrochloric acid, add 10 mL of alizarin red S TS, and then add water to make 30 mL.

**Zirconyl-alizarin S TS** See zirconyl-alizarin red S TS.

**Zirconyl nitrate** See zirconyl nitrate dihydrate.

**Zirconyl nitrate dihydrate**  $\text{ZrO}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$  A white crystalline powder. Freely soluble in water.

**Identification**—(1) To 5 mL of a solution (1 in 20) add 5 mL of sodium hydroxide TS: a white, milky precipitate is formed.

(2) To 10 mL of a solution (1 in 20) add 10 mL of sulfuric acid, cool, and superimpose 2 mL of iron (II) sulfate TS: a brown ring is produced at the zone of contact.

**Zolpidem tartrate for assay**  $(\text{C}_{19}\text{H}_{21}\text{N}_3\text{O})_2 \cdot \text{C}_4\text{H}_6\text{O}_6$  [Same as the monograph Zolpidem Tartrate. It contains not less than 99.5% of zolpidem tartrate  $[(\text{C}_{19}\text{H}_{21}\text{N}_3\text{O})_2 \cdot \text{C}_4\text{H}_6\text{O}_6]$ , calculated on the anhydrous basis.]

## 9.42 Solid Supports/Column Packings for Chromatography

**Aminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Carbamoyl group bound silica gel for liquid chromatography** Prepared for liquid chromatography.

**Cellulose for thin-layer chromatography** Use a high-grade cellulose prepared for thin-layer chromatography.

**Cellulose with fluorescent indicator for thin-layer chromatography** Use cellulose for thin-layer chromatography containing a suitable fluorescent substance.

**Cyanopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**DEAE-cross-linking dextran anion exchanger (Cl type), slightly alkaline** Slightly alkaline anion exchanger prepared by introducing diethylaminoethyl group into cross-linking dextran of gel filtration carrier.

**Diethylaminoethyl cellulose for column chromatography**

Prepared for column chromatography.

**Diethylaminoethyl group bound to synthetic polymer for liquid chromatography** Produced by binding diethylaminoethyl group to a hydrophilic synthetic polymer, for liquid chromatography. Exchange volume is about 0.1 mg equivalents/cm<sup>3</sup>.

**Dimethylaminopropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography** Dimethylsilanized silica gel for thin-layer chromatography to which a fluorescent indicator is added.

**Diol silica gel for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-methacrylate co-polymer for liquid chromatography** Prepared for liquid chromatography.

**Divinylbenzene-N-vinyl pyrrolidone copolymer for column chromatography** Prepared for column chromatography.

**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.

**Glycol etherified silica gel for liquid chromatography** Glycol group is bound to silica gel for liquid chromatography.

**Graphite carbon for gas chromatography** Prepared for gas chromatography.

**Hexasilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Hydrophilic silica gel for liquid chromatography** Diolized porous silica gel prepared for liquid chromatography (5–10 μm in particle diameter).

**Hydroxypropylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Neutral alumina for chromatography** Prepared for chromatography (75 – 180 μm in particle diameter).

**Neutral alumina for column chromatography** Prepared for column chromatography.

**Octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octadecylsilanized silica gel for thin-layer chromatography** Octadecylsilanized silica gel prepared for thin-layer chromatography.

**Octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography** Octadecylsilanized silica gel for thin-layer chromatography containing fluorescent indicator.

**Octadecylsilanized silicone polymer coated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Octylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Pentaethylenhexaaminated polyvinyl alcohol polymer bead for liquid chromatography** Prepared for liquid chromatography.

**Phenylated silica gel for liquid chromatography** Prepared for liquid chromatography.

**Phenylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Polyamide for column chromatography** Prepared for column chromatography.

**Polyamide for thin-layer chromatography** Prepared for thin-layer chromatography.

**Polyamide with fluorescent indicator for thin-layer chromatography** Add a fluorescent indicator to polyamide for thin-layer chromatography.

**Porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore diameter: 0.06 – 0.08  $\mu\text{m}$ , 100 – 200  $\text{m}^2/\text{g}$ )** A porous acrylonitrile-divinylbenzene copolymer prepared for gas chromatography.

**Porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography** Prepared for gas chromatography.

**Porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0075  $\mu\text{m}$ , 500 – 600  $\text{m}^2/\text{g}$ )** A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075  $\mu\text{m}$ , and surface area is 500 to 600  $\text{m}^2$  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.

**Porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085  $\mu\text{m}$ , 300 – 400  $\text{m}^2/\text{g}$ )** A porous styrene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0085  $\mu\text{m}$ , and surface area is 300 to 400  $\text{m}^2/\text{g}$ .

**Porous styrene-divinylbenzene copolymer for liquid chromatography** A porous styrene-divinylbenzene copolymer prepared for liquid chromatography.

**Quaternary alkylaminated styrene-divinylbenzene copolymer for liquid chromatography** 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 liquid chromatography with attached carbamoyl groups** Prepared for liquid chromatography.

**Silica gel for thin-layer chromatography** A silica gel pre-

pared for thin-layer chromatography.

**Silica gel for thin-layer chromatography (particle size 5 – 7  $\mu\text{m}$ , with fluorescent indication)** Prepared for high-performance thin-layer chromatography.

**Silica gel with complex fluorescent indicator for thin-layer chromatography** A silica gel for thin-layer chromatography containing suitable complex fluorescent indicators.

**Silica gel with fluorescent indicator for thin-layer chromatography** A silica gel for thin-layer chromatography containing a suitable fluorescent indicator.

**Siliceous earth for chromatography** A siliceous earth prepared for chromatography.

**Siliceous earth for gas chromatography** A siliceous earth prepared for gas chromatography.

**Slightly acidic ion-exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Strongly acidic ion-exchange resin for column chromatography** Prepared for column chromatography.

**Strongly acidic ion-exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Strongly acidic ion-exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Strongly basic ion-exchange resin for column chromatography** Prepared for column chromatography.

**Styrene-divinylbenzene copolymer for liquid chromatography** Prepared for liquid chromatography.

**Sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography** Prepared for column chromatography.

**Synthetic magnesium silicate for column chromatography** Prepared for column chromatography (150 – 250  $\mu\text{m}$  in particle diameter).

**Teflon for gas chromatography** See Tetrafluoroethylene polymer for gas chromatography.

**Terephthalic acid for gas chromatography**  $\text{C}_6\text{H}_4(\text{COOH})_2$  Prepared for gas chromatography.

**Tetrafluoroethylene polymer for gas chromatography** Prepared for gas chromatography.

**Trimethylsilanized silica gel for liquid chromatography** Prepared for liquid chromatography.

**Weakly acidic CM-bridged cellulose cation exchanger (H type)** Weakly acidic cation exchanger, intensified by crosslinking porous spherical cellulose, into which carboxymethyl groups have been introduced.

**Weakly acidic ion exchange resin for liquid chromatography** Prepared for liquid chromatography.

**Weakly acidic ion exchange silica gel for liquid chromatography** Prepared for liquid chromatography.

**Zeolite for gas chromatography (0.5 nm in pore diameter)** Zeolite prepared for gas chromatography.

## 9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.

**Filter paper** [R 3801, Filter paper (for chemical analysis), Filter paper for qualitative analysis]

No.1: For bulky gelatinous precipitate

No.2: For moderate-sized precipitate

No.3: For fine precipitate

No.4: Hardened filter paper for fine precipitate

**Filter paper for quantitative analysis** [R 3801, Filter paper (for chemical analysis), Filter paper for quantitative analysis]

No. 5A: For bulky gelatinous precipitate

No. 5B: For moderate-sized precipitate

No. 5C: For fine precipitate

No. 6: Thin filter paper for fine precipitate

**Porcelain crucible** [R 1301, Porcelain crucible for chemical analysis]

**Sintered glass filter** [R 3503, Glass appliance for chemical analysis, Buchner funnel glass filter]

G3: 20–30  $\mu\text{m}$  in pore size

G4: 5–10  $\mu\text{m}$  in pore size

**Blue litmus paper** See litmus paper, blue.

**Congo red paper** Immerse filter paper in congo red TS, and air-dry.

**Glass fiber** See glass wool.

**Glass wool** [K 8251, Special class]

**Lead acetate paper** See lead (II) acetate paper.

**Lead (II) acetate paper** Usually, immerse strips of filter paper, 6 cm  $\times$  8 cm in size, in lead (II) acetate TS, drain off the excess liquid, and dry the paper at 100°C, avoiding contact with metals.

**Litmus paper, blue** [K 9071, Litmus paper, Blue litmus paper]

**Litmus paper, red** [K 9071, Litmus paper, Red litmus paper]

**Phosgene test paper** Dissolve 5 g of 4-dimethylamino-benzaldehyde and 5 g of diphenylamine in 100 mL of ethanol (99.5). Immerse a filter paper 5 cm in width in this solution, and allow to dry spontaneously while the paper is suspended in a dark place under clear air. Then cut off the 5-cm portions from the upper side and lower side of the paper, and cut the remaining paper to a length of 7.5 cm.

Preserve in tight, light-resistant containers. Do not use the paper, which has changed to a yellow color.

**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-starch paper** Impregnate filter paper

with freshly prepared potassium iodide-starch TS, and dry in a clean room.

*Storage*—Store in a glass-stoppered bottle, protected from light and moisture.

**Red litmus paper** See litmus paper, red.

**Turmeric paper** Macerate 20 g of powdered dried rhizome of *Curcuma longa* Linné with four 100 mL-portions 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 100 mL 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 1 mL of hydrochloric acid and 4 mL 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.

**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.

## 9.44 Standard Particles, etc.

**$\alpha$ -Alumina for specific surface area determination**  
 $\alpha$ -Al<sub>2</sub>O<sub>3</sub> Prepared for specific surface area determination.

**$\alpha$ -Alumina for thermal analysis**  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> Prepared for thermal analysis.

**Calibration ball for particle density measurement**  
Calibration ball with a known volume prepared for measurement of particle density. The volume of the calibration ball must be accurately determined to the nearest 0.001 cm<sup>3</sup>.

**Indium for thermal analysis** In Prepared for thermal analysis.

*Content*: not less than 99.99%.

**Nickel for thermal analysis** [K 9062 (Nickel), Special class. *Content*: not less than 99.99%]

**Standard particles for calibrating light-shielded automatic fine particle counter** Use plastic spherical particles of known size and number.

**Tin for thermal analysis** Sn [K 8580 (Tin). *Content*: not less than 99.99%]

## Measuring Instruments and Appliances, Thermometers, etc.

### 9.61 Optical Filters for Wavelength and Transmission Rate Calibration

Use optical filters for wavelength calibration and those for transmission rate calibration shown in Table 9.61-1 and Table 9.61-2, respectively. The optical filters for transmission rate calibration are also used for the calibration of absorbances.

**Table 9.61-1** Optical Filters for Wavelength Calibration

Type of filter	Range of wavelength calibration (nm)	Product name
Neodymium optical filter for wavelength calibration	400 - 750	JCRM 001
Holmium optical filter for wavelength calibration	250 - 600	JCRM 002

**Table 9.61-2** Optical Filters for Transmission Rate Calibration

Type of filter	Transmission rate for calibration (%)	Product name
Optical filter for calibration within the visible wavelength range	1	JCRM 101
	10	JCRM 110
	20	JCRM 120
	30	JCRM 130
	40	JCRM 140
Optical filter for calibration within the ultraviolet wavelength range	50	JCRM 150
	10	JCRM 210 A
Optical filter for calibration within the near-ultraviolet wavelength range	50	JCRM 250 A
	10	JCRM 310
	30	JCRM 330
	50	JCRM 350

### 9.62 Measuring Instruments, Appliances

Measuring Instruments are the instruments or machines used for measuring mass or volume in the JP tests, and Appliances are the instruments specified in order to make test conditions as consistent as possible in those tests.

**Balances and weights** (1) Chemical balances—Use balances readable to the extent of 0.1 mg.

(2) Semimicrobalances—Use balances readable to the extent of 10  $\mu$ g.

(3) Microbalances—Use balances readable to the extent of 1  $\mu$ g.

(4) Weights—Use calibrated weights.

**Carbon dioxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon dioxide.

**Carbon monoxide measuring detector tube** [Gas detector tube measurement system K 0804] Packed with measurement packing for carbon monoxide.

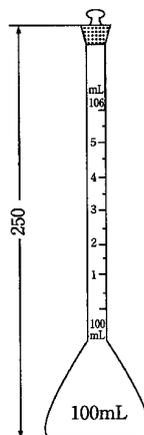
**Cassia flask** Use glass-stoppered flasks, shown in Fig. 9.62-1, made of hard glass and having graduation lines of volume on the neck.

**Gas mixer** Use the apparatus, shown in Fig. 9.62-3, made of hard glass.

**Nessler tube** Use colorless, glass-stoppered cylinders 1.0 to 1.5 mm in thickness, shown in Fig. 9.62-2, made of hard glass. The difference of the height of the graduation line of 50 mL from the bottom among cylinders does not exceed 2 mm.

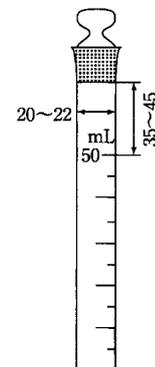
**Sieves** Sieves conform to the specifications in Table 9.62-1. Use the sieve number of nominal size as the designation.

**Volumetric measures** Use volumetric flasks, transfer pipets, push-button micropipets, burets and measuring cylinders conforming to the Japanese Industrial Standard.



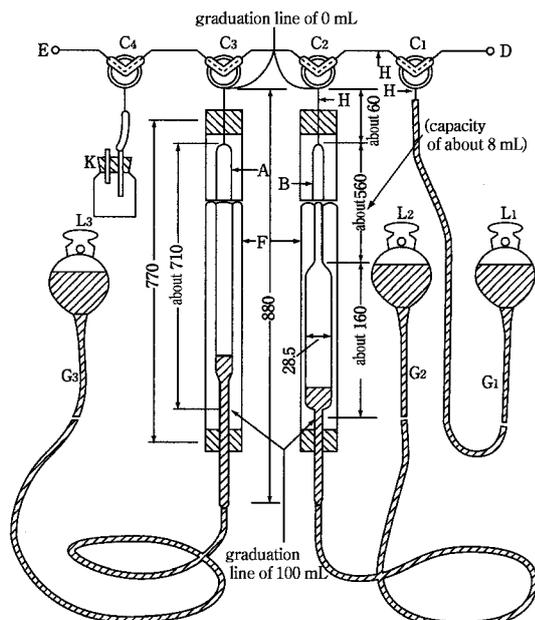
The figures are in mm.

**Fig. 9.62-1**



The figures are in mm.

**Fig. 9.62-2**



The figures are in mm.

- A: Gas buret (capacity of 100 mL, about 13.7 mm in inside diameter, graduated in 0.2 mL divisions, and graduated in 0.1 mL divisions at the lower, narrow part).
- B: Gas buret (capacity of 100 mL, about 4.2 mm in inside diameter at the upper stem with graduation in 0.02-mL division, about 28.5 mm in inside diameter at the lower stem with graduation in 1-mL divisions).
- C: (C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> and C<sub>4</sub>): 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<sub>1</sub>: about 80 cm in length; G<sub>2</sub> and G<sub>3</sub>: about 120 cm in length).
- H: Heavy-wall capillary tube (about 1 mm in inside diameter).
- K: Receiver.
- L: Leveling bulb (L<sub>1</sub>: filled with about 50 mL of mercury; L<sub>2</sub> and L<sub>3</sub>: filled with about 150 mL of mercury).

Fig. 9.62-3

## 9.63 Thermometers

**Thermometers** Ordinarily, use calibrated thermometers with an immersion line (rod) or calibrated total immersion mercury-filled thermometers according to the Japanese Industrial Standards. Use the thermometers with the immersion line (rod), shown in Table 9.63-1, for the tests in Congealing Point, Melting Point (Method 1), Boiling Point and Distilling Range.

Table 9.62-1 Specification of Sieves

Sieve number	Nominal size ( $\mu\text{m}$ )	Nominal opening (mm)	Permissible variation of opening (mm)		Diameter of wire (mm)		
			Average	Maximum	Recommended	Maximum	Minimum
3.5	5600	5.60	$\pm 0.18$	0.47	1.60	1.90	1.30
4	4750	4.75	$\pm 0.15$	0.41	1.60	1.90	1.30
4.7	4000	4.00	$\pm 0.13$	0.37	1.40	1.70	1.20
5.5	3350	3.35	$\pm 0.11$	0.32	1.25	1.50	1.06
6.5	2800	2.80	$\pm 0.09$	0.29	1.12	1.30	0.95
7.5	2360	2.36	$\pm 0.08$	0.25	1.00	1.15	0.85
8.6	2000	2.00	$\pm 0.07$	0.23	0.90	1.04	0.77
10	1700	1.70	$\pm 0.06$	0.20	0.80	0.92	0.68
12	1400	1.40	$\pm 0.05$	0.18	0.71	0.82	0.60
14	1180	1.18	$\pm 0.04$	0.16	0.63	0.72	0.54
16	1000	1.00	$\pm 0.03$	0.14	0.56	0.64	0.48
18	850	0.850	$\pm 0.029$	0.127	0.500	0.580	0.430
22	710	0.710	$\pm 0.025$	0.112	0.450	0.520	0.380
26	600	0.600	$\pm 0.021$	0.101	0.400	0.460	0.340
30	500	0.500	$\pm 0.018$	0.089	0.315	0.360	0.270
36	425	0.425	$\pm 0.016$	0.081	0.280	0.320	0.240
42	355	0.355	$\pm 0.013$	0.072	0.224	0.260	0.190
50	300	0.300	$\pm 0.012$	0.065	0.200	0.230	0.170
60	250	0.250	$\pm 0.0099$	0.058	0.160	0.190	0.130
70	212	0.212	$\pm 0.0087$	0.052	0.140	0.170	0.120
83	180	0.180	$\pm 0.0076$	0.047	0.125	0.150	0.106
100	150	0.150	$\pm 0.0066$	0.043	0.100	0.115	0.085
119	125	0.125	$\pm 0.0058$	0.038	0.090	0.104	0.077
140	106	0.106	$\pm 0.0052$	0.035	0.071	0.082	0.060
166	90	0.090	$\pm 0.0046$	0.032	0.063	0.072	0.054
200	75	0.075	$\pm 0.0041$	0.029	0.050	0.058	0.043
235	63	0.063	$\pm 0.0037$	0.026	0.045	0.052	0.038
282	53	0.053	$\pm 0.0034$	0.024	0.036	0.041	0.031
330	45	0.045	$\pm 0.0031$	0.022	0.032	0.037	0.027
391	38	0.038	$\pm 0.0029$	0.020	0.030	0.035	0.024

Table 9.63-1 Thermometers with Immersion Line

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon
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	1°C
Graduation numbered at	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C	2°C
Total length (mm)	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300
Stem diameter (mm)	6.0±0.3	6.0±0.3	6.0±0.3	6.0±0.3	6.0±0.3	6.0±0.3
Bulb length (mm)	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18
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 - 65	35 - 65	35 - 65	35 - 65	35 - 65	35 - 65
Distance from bottom of bulb to immersion line(mm)	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62
From of top of thermometer	loop	loop	loop	loop	loop	loop
Test temperature	-15°C, 15°C, 45°C	45°C, 70°C, 95°C	95°C, 120°C, 145°C	145°C, 170°C, 195°C	195°C, 220°C, 245°C	245°C, 280°C, 315°C
Maximum scale error at any point	0.2°C	0.2°C	0.2°C	0.2°C	195°C : 0.2°C 220°C : 0.3°C 245°C : 0.3°C	245°C : 0.3°C 280°C : 0.4°C 315°C : 0.5°C