C: A marked line.
D: Bath made of glass or plastics.
E: Stirring rod made of glass or stainless steel (3 mm in diameter, the lower end part of it is bent to make a loop, about 18 mm in diameter).
F: Thermometer with an immersion line.
G: Thermometer with an immersion line or a total immersion thermometer.
H: Immersion line

Procedure

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

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

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

10. Content Uniformity Test

Content Uniformity Test is the test to determine the uniformity of dosage units by assay of individual units as directed in the individual monograph. If no specification for the *Content Uniformity Test* exists in the monograph, use the method in individual *Assay* procedure or an alternative appropriate method. Apply the following test unless otherwise specified in the individual monograph.

Select 30 units, assay the first 10 units individually and calculate the acceptance value. The requirements are met if the acceptance value is less than or equal to 15.0%. When the acceptance value is greater than 15.0%, test the next 20 units. The requirements are met if the final acceptance value of the 30 dosage units does not exceed 15.0% and no unit shows a deviation that exceeds 25.0% of the label claim.

$$\text{Acceptance value} = |M - \bar{X}| + ks$$

M: Label claim (100.0%), unless otherwise specified in the individual monograph.
$\bar{X}$: Mean of individual contents (x_1, $x_2 \cdots x_n$).
x_1, $x_2 \cdots x_n$: Individual contents of the units tested, expressed as a percentage of the label claim.
n: Sample size (number of units in a sample).
k: Acceptability constant, $k = 2.2$ when the sample size is 10, and $k = 1.9$ when the sample size is 30.
s: Standard deviation of the sample.

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{X})^2}{n - 1}}$$

11. Crude Drugs Test

The Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

Sampling

Unless otherwise specified, sample should be taken by the following methods. If necessary, preserve the samples in tight containers.

(1) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(2) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.

(3) 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.

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.

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.

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 (%).

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 cruci-

ble, 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.

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.

Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

(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 intermittent 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.

(2) Water-soluble extract—Proceed as directed in (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.

(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 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 (%).

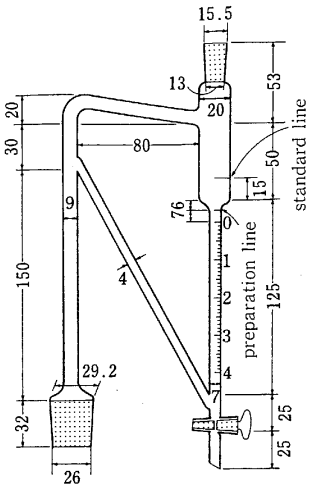


Fig. 1

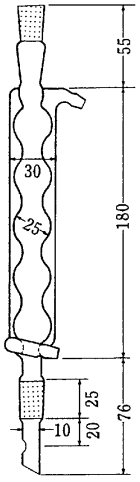


Fig. 2

The figures are in mm.

Essential oil content

The test of essential oil content in crude drugs is performed as directed in the following method:

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. 1), inserting a reflux condenser (Fig. 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.

Microscopic examination

(1) Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

(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 μm in thickness.

(ii) Powder: Place about 0.1 g of powdered sample in a watch glass containing 2 to 3 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for more than 10 minutes to swell the sample. Smear, using a samll glass rod, the slide glass with a small amount of the swollen 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.

Unless otherwise specified, use a mixture of glycerin and water (1:1) as mounting agent and swelling agent.

(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.

12. Determination of Specific Gravity and Density

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

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

Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 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 W. 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' °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 W_1 of the pycnometer filled with the sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass W_2 at the specified temperature t°C. The specific gravity $d_t^{t'}$ can be calculated by use of the following equation.

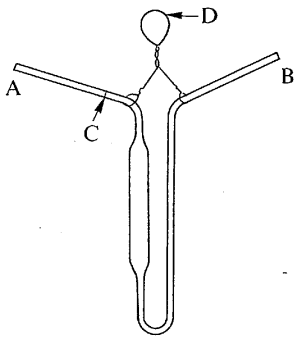
$$d_t^{t'} = \frac{W_1 - W}{W_2 - W}$$

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

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

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 the figure, both ends are thick-walled fine tubes (inside diameter: 1 - 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, W, 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°C to 5°C than the specified temperature t'°C. Attach rubber tubing or a ground-glass tube to the end of A, and suck up the 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'°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 W_1. By use of the same pycnometer, perform the same procedure for the standard solution of water. Weigh the pycnometer containing water at the specified temperature t°C, and note the mass W_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' °C = t°C), the density of sample solution at temperature t' °C can be calculated by using the equation described in Method 1.

Method 3. Masurement 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' °C and the hydrometer comes to a standstill, read the specific gravity $d_t^{t'}$ or the density $\rho_T^{t'}$ at the upper brim of the meniscus. Here the temperature t°C indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a sample solution is performed at the same temperature (t' °C = t°C), at which the hydrometer is calibrated, the density of a sample solution at t' °C, $\rho_T^{t'}$, can be calculated by using the specific gravity $d_t^{t'}$ and the equation shown in Method 1.

Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period T (s) of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period T in proportion to the mass of the sample