as the sample solution. Separately, dissolve 0.05 g of Morphine Hydrochloride in 10 mL of a mixture of water and ethanol (99.5) (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution is the same in color tone and *Rf* value with that of the standard solution (morphine).

(2) To 10 mL of Morphine and Atropine Injection add 7 mL of sodium hydroxide TS, add sodium chloride to saturate, and extract with two 10-mL portions of chloroform. Evaporate the chloroform extract on a water bath to dryness, add 5 drops of fuming nitric acid to the residue, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops (atropine).

Assay (1) Morphine hydrochloride—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$)
= amount (mg) of morphine hydrochloride for assay, calculated on anhydrous basis

$$\times \frac{Q_T}{Q_S} \times 1.1680$$

Internal standard solution—A solution of Etilefrine Hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of morphine and the internal standard in this order with the resolution between these peaks being not less than 3.

(2) Atropine sulfate—Pipet 2 mL of Morphine and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 0.03 g of atropine sulfate for assay (separately determine its loss on drying in the same manner as directed under Atropine Sulfate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then proceed in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of atropine to that of the internal standard.

Amount (mg) of atropine sulfate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
= amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

$$\times \frac{Q_T}{Q_S} \times \frac{1}{50} \times 1.207$$

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 1.5 m in length, packed with 180- to 250-μm siliceous earth for gas chromatography coated with 1 to 3% of methylphenylsilicone polymer.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium

Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Moutan Bark

Moutan Cortex

ボタンピ

Moutan Bark is the root bark of *Paeonia suffruticosa* Andrews (*Paeonia moutan* Sims) (*Paeoniaceae*).

It contains not less than 1.0% of paeonol.

Description Tubular to semi-tubular bark, about 0.5 cm in thickness, 5 - 8 cm in length, 0.8 - 1.5 cm in diameter; externally dark brown to purplish brown, with small and transversely elongated ellipsoidal scars of lateral roots, and with longitudinal wrinkles; internally, light grayish brown to purplish brown and smooth; fractured surface coarse; white crystals often attached on the internal and fractured surfaces. Odor, characteristic; taste, slightly pungent and bitter.

Identification To 2.0 g of pulverized Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 10 ml of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution and the standerd solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among several spots from the sample solution is similar with the spot from the standard solution in color tone and *Rf* value.

Purity **(1)** Xylem—The amount of the xylem contained in Moutan Bark does not exceed 5.0%.

(2) Foreign matter—The amount of foreign matter other than xylem contained in Moutan Bark does not exceed 1.0%.

Total ash Not more than 6.0%.

Acid-insoluble ash Not more than 1.0%.

Component determination Weigh accurately about 0.3 g of pulverized Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, dry paeonol for component determination in a desiccator (calcium chloride for dring) for more than 1 hour. Weigh accurately about 0.01 g of it, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of paeonol in each solution.

$$\text{Amount (mg) of paeonol} = \text{amount (mg) of paeonol for component determination} \times \frac{A_T}{A_S} \times \frac{1}{2}$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

Selection of column: Dissolve 0.001 g of paeonol for component determination and 0.005 g of butyl parahydroxybenzoate in 25 mL of methanol. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of paeonol and butyl parahydroxybenzoate in this order, with the resolution between these peaks being not less than 2.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of paeonol is not more than 1.5%.

Powdered Moutan Bark

Moutan Cortex Pulveratus

ボタンピ末

Powdered Moutan Bark is the powder of Moutan Bark.

It contains not less than 0.7% of paeonol.

Description Powdered Moutan Bark occurs as a light grayish yellow-brown powder. It has a characteristic odor and a slight, pungent and bitter taste.

Under a microscope, Powdered Moutan Bark reveals starch grains and fragments of parenchyma containing them; fragments of cork tissue containing tannin; fragments of somewhat thick-walled collenchyma, medullary rays, and phloem parenchyma; rosette aggregates of calcium oxalate and also fragments of parenchyma cells containing them. Starch grains are simple or 2- to 10-compound grains, 10 - 25 μm in diameter; rosette aggregates are 20 - 30 μm in diameter.

Identification To 2.0 g of Powdered Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1mg of peonol for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among several spots from the sample solution is similar with the spot from the standard solution in color tone and *Rf* value.

Purity Foreign matter—Under a microscope, usually vessels and other thick-walled cells are not observable.

Total ash Not more than 6.0%.

Acid-insoluble ash Not more than 1.0%.

Component determination Weigh accurately about 0.5 g of Powdered Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol