Phenol Spirit: a dark blue-purple color develops (iodine).

(2) To 1 mL of Iodine, Salicylic Acid and Phenol Spirit add 5 mL of ethanol (95) and water to make 50 mL. To 1 mL of this solution add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 15 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 25 mL of diethyl ether. Wash the diethyl ether extract with two 25-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is developed (phenol).

(4) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 10 mL of diethyl ether, and use the diethyl ether extract as the sample solution. Dissolve 0.025 g of salicylic acid, 0.01 g of phenol and 0.04 g of benzoic acid in 5 mL each of diethyl ether, respectively, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with the sample solution and the standard solutions as directed under the Thin-layer Chromatography. Spot 5 μL of each solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the 3 spots from the sample solution show the same Rf value as the corresponding spots of the standard solutions (1), (2) and (3). Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution acquires a purple color.

Assay (1) Iodine—Pipet 4 mL of Iodine, Salicylic Acid and Phenol Spirit, add ethanol (95) to make exactly 50 ml, and use this solution as the sample solution. On the other hand, weigh accurately about 1.2 g of iodine for assay and about 0.8 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 ml. Pipet 4 ml of this solution, add ethanol (95) to make exactly 50 ml, and use this solution as the standard solution. Pipet 3 ml each of the sample solution and the standard solution, to each add exactly 25 ml of a mixture of chloroform and hexane (2:1), and shake. Further add exactly 10 ml of water, shake and separate the chloroform-hexane layers [use the water layers in (2)]. Filter through a pledget of absorbent cotton, and determine the absorbances of the filtrates from the sample solution and the standard solution, respectively, A_T and A_S, at 512 nm as directed under the Ultraviolet-visible Spectrophotometry, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = \text{amount (mg) of iodine for assay} \times \frac{A_T}{A_S} \times \frac{1}{25}$$

(2) Potassium iodide—Separate the water layers of the sample solution and the standard solution obtained in the Assay (1), pipet 8 ml each of the water layers, and add 1 ml of diluted dilute hydrochloric acid (1 in 2) and 1 ml of sodium nitrite TS. Immediately after shaking, add exactly 10 ml of a mixture of chloroform and hexane (2:1), shake, and proceed in the same manner as for the Assay (1).

$$\text{Amount (mg) of potassium iodide (KI)} = \text{amount (mg) of potassium iodide for assay} \times \frac{A_T}{A_S} \times \frac{1}{25}$$

(3) Salicylic acid, phenol and benzoic acid—Pipet 2 mL of Iodine, Salicylic Acid and Phenol Spirit, add 20 mL of diluted methanol (1 in 2) and 0.1 mol/L soium thiosulfate VS until the color of iodine disappears, add exactly 20 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 200 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, about 0.08 g of phenol for assay, and 0.32 g of benzoic acid, previously dried in a desiccator (silica gel) for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 20 mL of the internal standard solution and diluted methanol (1 in 2) to make 200 mL, and use this solution as the standard solution. Perform the test with 3 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the operating conditions in the Assay under Compound Salicylic Acid Spirit. Calculate the ratios, Q_{Ta}, Q_{Tb} and Q_{Tc}, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the sample solution, and the ratios, Q_{Sa}, Q_{Sb} and Q_{Sc}, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the standard solution.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = \text{amount (mg) of salicylic acid for assay} \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{2}$$

$$\text{Amount (mg) of phenol } (C_6H_6O) = \text{amount (mg) of phenol for assay} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{2}$$

$$\text{Amount (mg) of benzoic acid } (C_7H_6O_2) = \text{amount (mg) of benzoic acid} \times \frac{Q_{Tc}}{Q_{Sc}} \times \frac{1}{2}$$

Internal standard solution—A solution of theophylline in methanol (1 in 1000).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ipecac

Ipecacuanhae Radix

トコン

Ipecac is the root and rhizome of *Cephaelis ipecacuanha* (Broterol) A. Richard or *Cephaelis acuminata* Karsten (*Rubiaceae*).

It contains not less than 2.0% of the total alkaloids

(emetine and cephaeline), calculated on the basis of dried material.

Description Slender, curved, cylindrical root, 3 - 15 cm in length, 0.3 - 0.9 cm in diameter; mostly twisted, and sometimes branched; outer surface gray, dark grayish brown, reddish brown in color and irregularly annulated; when root fractured, cortex easily separable from the xylem; the cortex on the fractured surface is grayish brown, and the xylem is light brown in color: thickness of cortex up to about two-thirds of radius in thickened portion. Scales in rhizome opposite. Odor, slight; powder irritates the mucous membrane of the nose; taste, slightly bitter and unpleasant.

Under a microscope, the transverse section of Ipecac reveals a cork layer, consisting of brown thin-walled cork cells; in the cortex, sclerenchyma cells are absent; in the xylem, vessels and tracheids arranged alternately; parenchyma cells filled with starch grains and sometimes with raphides of calcium oxalate.

Identification To 0.5 g of pulverized Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small pieces of chlorinated lime: circumference of it turns red.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 5.0%.

Acid-insoluble ash Not more than 2.0%.

Component determination Weigh accurately about 0.5 g of pulverized Ipecac, in a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of emetine hydrochloride for component determination, previously dried in a desiccator (reduced below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μL of the sample solution and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_{TE} and A_{TC}, of emetine and cephaeline in the sample solution, and the peak area, A_{SE}, of emetine in the standard solution.

$$\text{Amount (mg) of total alkaloids (emetine and cephaeline)} = \text{amount (mg) of emetine hydrochloride for component determination} \times \frac{A_{TE} + A_{TC} \times 0.971}{A_{SE}} \times 0.868$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength : 283 nm).

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

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

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

Selection of column: Dissolve 1 mg each of emetine hydrochloride for component determination and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μL of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly dividing each peak.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of emetine is not more than 1.5%.

Powdered Ipecac

Ipecacuanhae Radix Pulverata

トコン末

Powdered Ipecac is the powder of Ipecac or its powder diluted with Potato Starch.

It contains not less than 2.0% and not more than 2.6% of the total alkaloids (emetine and cephaeline).

Description Powdered Ipecac occurs as a light grayish yellow to light brown powder. It has a slight odor, which is irritating to the nasal mucosa, and has a somewhat bitter and unpleasant taste.

Under a microscope, Powdered Ipecac reveals starch grains and needle crystals of calcium oxalate; fragments of parenchyma cells containing starch grains or the needle crystals; substitute fibers,thin-walled cork tissue; vessels and tracheids with simple or bordered pits; a few wood fibers and wood parenchyma. Starch grains inherent in Ipecac, mainly 2 - 8-compound grains, rarely simple grains 4 - 22 μm in diameter; and needle crystals of calcium oxalate 25 - 60 μm in length.

Identification To 0.5 g of Powdered Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small pieces of chlorinated lime: circumference of it turns red.

Purity Foreign matter—Under a microscope, groups of stone cells and thick-walled fibers are not observed.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 5.0%.

Acid-insoluble ash Not more than 2.0%.

Component determination Weigh accurately about 0.5 g of Powdered Ipecac, transfer into a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine