on drying before use), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 0.025 g of Scopolamine Hydrobromide Reference Standard (determine the loss on drying before use), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of standard stock solution A and 1 mL of standard stock solution B, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, 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. Determine the ratios, Q_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine), and the ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

Amount (mg) of hyoscyamine ($C_{17}H_{23}NO_3$)
= amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis
$\times \frac{Q_{TA}}{Q_{SA}} \times \frac{1}{5} \times 0.855$

Amount (mg) of scopolamine ($C_{17}H_{21}NO_4$)
= amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis
$\times \frac{Q_{TS}}{Q_{SS}} \times \frac{1}{25} \times 0.789$

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

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

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

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to a pH of 3.5, and add water to make 1000 mL. To 9 parts of this solution add 1 part of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and the resolution between the peaks of atropine and the internal standard being not less than 4.

Compound Scopolia Extract and Tannic Acid Suppositories

複方ロートエキス・タンニン坐剤

Method of preparation

Scopolia Extract	0.2 g
Tannic Acid	0.3 g
Ichthammol	2.0 g
Ethyl Aminobenzoate	1 g
Cacao Butter or a suitable base	a sufficient quantity

Prepare 10 suppositories as directed under Suppositories with the above ingredients.

Description Compound Scopolia Extract and Tannic Acid Suppositories are blackish brown suppositories, having a characteristic odor.

Identification **(1)** Shake 2 Compound Scopolia Extract and Tannic Acid Suppositories with 20 mL of diethyl ether for 10 minutes to dissolve the base of suppositories. Shake thoroughly the mixture with 15 mL of water, separate the water layer, and filter. To the filtrate add 10 mL of chloroform, shake well, and separate the chloroform layer. Take 5 mL of the chloroform solution, add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

(3) To 2 Compound Scopolia Extract and Tannic Acid Suppositories add 10 mL of hot water, heat on a water bath for 10 minutes with occasional stirring, and cool in ice. Remove the coagulation on the solution with a glass rod, and filter. Boil 5 mL of the filtrate with 5 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue (ichthammol).

(4) To 1 suppository of Compound Scopolia Extract and Tannic Acid Suppositories add 40 mL of ethanol (95). Warm for 20 minutes on a water bath with stirring. Cool in ice, centrifuge, and filter the supernatant liquid. To 1 mL of the filtrate add 4 mL of ethanol (95), and use this solution as the sample solution. Dissolve 0.025 g of ethyl aminobenzoate in 50 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 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 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *Rf* value.

Containers and storage Containers—Well-closed containers.