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Ryokeijutsukanto Extract

茶桂朮甘湯エキス

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, and not less than 21 mg and not more than 63 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Poria Sclerotium	6 g	6 g
Cinnamon Bark	4 g	4 g
Atractylodes Rhizome	3 g	—
Atractylodes Lancea Rhizome	—	3 g
Glycyrrhiza	2 g	2 g

Prepare a dry extract or viscous extract as directed under

Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Ryokeijutsukanto Extract occurs as a brown powder or blackish brown viscous extract. It has an odor, and a sweet first then bitter taste.

Identification (1) To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid 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 5 μL 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 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 5 μL 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of dry extract (or 6.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, 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 μL 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 and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R_f* value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water,

shake, then add 10 mL of 1-butanol, and shake. Centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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 5 μL 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 and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) of Ryokeijutsukanto Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) of Ryokeijutsukanto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g 105°C, 5 hours).

Total ash <5.01> Not more than 8.0%, calculated on the dried basis.

Assay (1) (E)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance) of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (E)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) 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 the peak areas, A_T and A_S , of (E)-cinnamic acid in each solution.

$$\begin{aligned} &\text{Amount (mg) of (E)-cinnamic acid} \\ &= M_S \times A_T/A_S \times 1/20 \end{aligned}$$

M_S : Amount (mg) of (E)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 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 μm in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (E)-cinnamic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (E)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (E)-cinnamic acid is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance) of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) 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 the peak areas, A_T and A_S , of glycyrrhizic acid in each solution.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= M_S \times A_T/A_S \times 1/2 \end{aligned}$$

M_S : Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 μm in particle diameter).

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

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

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