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Shoseiryuto Extract

小青竜湯エキス

Shoseiryuto Extract contains not less than 10 mg and not more than 30 mg of the total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 26 mg and not more than 78 mg of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

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Method of preparation

	1)	2)
Ephedra Herb	3 g	3 g
Peony Root	3 g	3 g
Processed Ginger	3 g	—
Ginger	—	3 g
Glycyrrhiza	3 g	3 g
Cinnamon Bark	3 g	3 g
Asiasarum Root	3 g	3 g
Schisandra Fruit	3 g	3 g
Pinellia Tuber	6 g	6 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 Shoseiryuto Extract occurs as a light brown to blackish brown, powder or viscous extract. It has a characteristic odor and a acid first then pungent taste.

Identification (1) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ephedrine hydrochloride 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 μ L 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 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-purple spot from the standard solution (Ephedra Herb).

(2) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin RS 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 μ L 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 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution (Peony Root).

(3) For preparation prescribed Processed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol 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 20 μ L of the sample so-

lution and 1 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution (Processed Ginger).

(4) For preparation prescribed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingeol 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 10 μ L of the sample solution and 5 μ L of the 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 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution (Ginger).

(5) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, 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 μ L 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 TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. Previously, add water up to the base point line of the graduated tube of the apparatus, and then add 2 mL of hexane. After heating under reflux for about 1 hour, take the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde 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 20 μ L of the sample solution and 2 μ L the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot

among the several spots obtained from the sample solution has the same color tone and *R_f* value with the yellow-orange spot from the standard solution.

(ii) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde 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 20 μ L of the sample solution and 2 μ L the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the bluish white fluorescent spot from the standard solution.

(7) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of asarinin 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 20 μ L of the sample solution and 5 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) 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 obtained from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution (Asiasarum Root).

(8) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of schisandrin 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 10 μ L of the sample solution and 5 μ L of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) 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 obtained from the sample solution has the same color tone and *R_f* value with the blue-purple spot from the standard solution (Schisandra Fruit).

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

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract,

equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 10.0% (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 12.0%, calculated on the dried basis.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract equivalent to about 0.5 g of dried substance), 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 ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L 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_{TE} and A_{TP} , of ephedrine and pseudoephedrine from the sample solution, and the peak area, A_S , of ephedrine from the standard solution.

$$\begin{aligned} & \text{Amount (mg) of total alkaloids [ephedrine (C}_{10}\text{H}_{15}\text{NO}) \\ & \text{and pseudoephedrine (C}_{10}\text{H}_{15}\text{NO)]} \\ & = M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819 \end{aligned}$$

M_S : Amount (mg) of ephedrine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 μ m in particle diameter).

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

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

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

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), 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 Peoniflorin RS, (separately determined the

water), 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 μ L 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 peoniflorin in each solution.

$$\begin{aligned} &\text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ &= M_S \times A_T/A_S \times 1/2 \end{aligned}$$

M_S : Amount (mg) of Peoniflorin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 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 μ m in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, albiflorin and peoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

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

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), 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), 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 μ L 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, 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 μ m in particle diameter).

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

Mobile phase: A mixture of dilute 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 μ L 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 μ L 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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