JP XVIII (2021) JP XVIII (2021) JP XVIII (2021) JP XVIII (2021) JP XVIII (2021)

Goshuyuto Extract

呉茱萸湯エキス

Goshuyuto Extract contains not less than 0.3 mg (for preparation prescribed 3 g of Euodia Fruit) or not less than 0.4 mg (for preparation prescribed 4 g of Euodia Fruit) of evodiamine, not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 1 g of Ginger) or not less than 0.7 mg and not more than 2.8 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, and not less than 1.2 mg (for preparation prescribed 2 g of Ginseng) or not less than 1.8 mg (for preparation prescribed 3 g of Ginseng) of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Euodia Fruit	3 g	4 g	3 g
Ginger	1 g	1.5 g	1.5 g
Ginseng	2 g	3 g	2 g
Jujube	4 g	3 g	4 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above.

Description Goshuyuto Extract occurs as a light brown to light red-yellow powder, or a black-brown viscous extract. It has a slight odor and a hot and bitter taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, to 1 g of pulverized euodia fruit add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under

Thin-layer Chromatography <*2.03*>. Spot 1 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 acetone, 2-propanol, water and formic acid (7:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot (*R*f value: about 0.5) from the standard solution (Euodia Fruit).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 μL of the sample solution and 5 μL 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 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(3) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 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, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of 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 (Ginseng).

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 the dried substance) as directed under 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 the 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 11.0% (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 10.0%, calculated on the dried basis.

Assay **(1)** Evodiamine—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of evodiamine for assay, and dissolve in methanol to make exactly 200 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 evodiamine in each solution.

$$\text{Amount (mg) of evodiamine} = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of evodiamine for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 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 (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of evodiamine is about 18 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 evodiamine 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 evodiamine is not more than 1.5%.

(2) [6]-Gingerol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 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 [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in (1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 14 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 [6]-gingerol 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 operat-

ing conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

(3) Ginsenoside Rb_1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to about 2 g of the dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine all the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column [about 10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), and washed just before using with methanol and then diluted methanol (3 in 10)], and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. 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 exactly 20 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 ginsenoside Rb_1 in each solution.

Amount (mg) of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$)
$= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl group bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (400:100:1).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb_1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

Containers and storage Containers—Tight containers.

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