Goshuyuto Extract

Goshuyuto Extract contains not less than 0.3 mg (for preparation prescribed 3 g of Evodia Fruit) or not less than 0.4 mg (for preparation prescribed 4 g of Evodia Fruit) of evodiamine, and 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, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

- 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 supernatant liquid as the sample solution. Separately, to 1 g of pulverized evodia 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 ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dimethylaminobenzaldehyde TS on the plate, heat 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 Rf 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 supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rb1, 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 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 Rf 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 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 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.

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

**MC**: Amount (mg) of evodiamine for assay taken

**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 octadeclsiylized silica gel for liquid chromatography (5 µm 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 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 operating conditions, the relative standard deviation of the peak area of 6-gingerol is not more than 1.5%.

**Containers and storage** — Containers — Tight containers.

**Evodiamine for assay** C_{19}H_{17}N_{3}O White to light yellow crystals or crystalline powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** — Proceed as directed in the Assay: it exhibits a doublet-doublet-like signal equivalent to one proton around δ 2.82 ppm, signals equivalent to four protons which includes a singlet signal around δ 2.91 ppm and a multiplet signal around δ 2.90 ppm – δ 2.98 ppm, a doublet-like signal equivalent to one proton around δ 3.23 ppm, a doublet-like signal equivalent to one proton around δ 4.66 ppm, a singlet signal equivalent to one proton around δ 6.16 ppm, a triplet-like signal equivalent to one proton around δ 7.00 ppm, a triplet-like signal equivalent to one proton around δ 7.05 ppm, a doublet-like signal equivalent to one proton around δ 7.08 ppm, a triplet-like signal equivalent to one proton around δ 7.14 ppm, a doublet-like signal equivalent to one proton around δ 7.39 ppm, a doublet-like signal equivalent to one proton around δ 7.51 ppm, a multiplet-like signal equivalent to one proton around δ 7.52 ppm and a doublet-like signal equivalent to one proton around δ 7.83 ppm.

**Unity of peak** — Dissolve 1 mg of evodiamine for assay in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 10 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of evodiamine peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

**Operating conditions** —

**Column**, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in (1).

System suitability

System performance: When the procedure is run with 10 µL of the sample 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.

Assay—Weigh accurately 5 mg of evodiamine for assay and 1 mg of DSS-d₆ for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve them in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ¹H-NMR as directed under Operating conditions, using DSS-d₆ for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 6.16 ppm assuming the signal of the internal reference compound as δ 0 ppm.

Amount (%) of evodiamine \( (C_{19}H_{17}N_3O) \)

\[ = \frac{M \times I \times P}{(M \times N) \times 1.3521} \]

M: Amount (mg) of evodiamine for assay taken

Mₛ: Amount (mg) of DSS-d₆ for nuclear magnetic resonance spectroscopy taken

I: Signal resonance intensity A based on the signal resonance intensity of DSS-d₆ for nuclear magnetic resonance spectroscopy as 9.000

N: Number of the hydrogen derived from A

P: Purity (%) of DSS-d₆ for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectrometer having ¹H resonance frequency of not less than 400 MHz.

Target nucleus: ¹H.

Digital resolution: 0.25 Hz or lower.

Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

¹³C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 6.16 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 6.16 ppm is not overlapped with any signal of obvious foreign substance.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity A to that of the internal reference compound is not more than 1.0%.

Evodia fruit [Same as the namesake monograph]