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Mukoi-Daikenchuto Extract

無コウイ大建中湯エキス

Mukoi-Daikenchuto Extract contains not less than 1.8 mg of ginsenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29), and

not less than 1.4 mg and not more than 4.2 mg of [6]-shogaol, per extract prepared with the amount specified in the Method of preparation.

Method of preparation Prepare a dry extract as directed under Extracts, with 2 g of Zanthoxylum Fruit, 3 g of Ginseng and 5 g of Processed Ginger.

Description Mukoi-Daikenchuto Extract is a light brown powder. It has a slight odor, and has a pungent taste.

Identification (1) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, then shake with 10 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, pulverize zanthoxylum fruit, shake 2.0 g with 10 mL of water, then shake with 5 mL of diethyl ether, 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 10 μ L 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 ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) 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 dark purple spot (*R_f* value: about 0.3) from the standard solution (Zanthoxylum Fruit).

(2) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb₁ 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 10 μ L of the sample solution and 2 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, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS 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 purple spot from the standard solution (Ginseng).

(3) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 10 μ L of the sample solution and 2 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 (Processed ginger).

Purity (1) Heavy metals <1.07>—Prepare the test solution with 2.0 g of Mukoi-Daikenchuto Extract as directed under

Extracts (4), and perform the test (not more than 15 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Mukoi-Daikenchuto Extract according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying <2.41> Not more than 5.9% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%.

Assay (1) Ginsenoside Rb₁—Weigh accurately about 2 g of Mukoi-Daikenchuto Extract, 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 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, 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 [10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μ m 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 solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb₁ RS (separately determine the water), 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 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 ginsenoside Rb₁ in each solution.

$$\begin{aligned} & \text{Amount (mg) of ginsenoside Rb}_1 \text{ (C}_{54}\text{H}_{92}\text{O}_{23}) \\ & = M_S \times A_T / A_S \times 1/5 \end{aligned}$$

M_S: Amount (mg) of Ginsenoside Rb₁ RS, 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 μ m in particle diameter).

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

Mobile phase: A mixture of acetonitrile and water (4:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb₁ is about 16 minutes).

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb₁ are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb₁ is not more than 1.5%.

(2) [6]-Shogaol—Weigh accurately about 0.5 g of Mukoi-Daikenchuto Extract, add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of [6]-shogaol for assay, dissolve in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (3 in 4) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 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 [6]-shogaol in each solution.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T / A_S \times 1/10$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 0.1 g of oxalic acid dihydrate in 600 mL of water, and add 400 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of [6]-shogaol is about 30 minutes).

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Containers and storage Containers—Tight containers.

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