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Mukoi-Daikenchuto Extract

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Mukoi-Daikenchuto Extract contains not less than 1.8 mg of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 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

	1)
Japanese Zanthoxylum Peel	2 g
Ginseng	3 g
Processed Ginger	5 g

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

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, powder japanese zanthoxylum peel, 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 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 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 (Japanese Zanthoxylum Peel).

(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 supernatnt liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 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 μL of the sample solution and 2 μL 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 obtained 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 μL of the sample solution and 2 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 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 obtained 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 Rb_1—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 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 solution 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.

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

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 (the retention time of ginsenoside Rb_1 is about 16 minutes).

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%.

(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 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]-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 taken

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 μm 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 μL 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 μL 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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