Mashiningan Extract

麻子仁丸エキス

Mashiningan Extract contains not less than 14 mg and not more than 56 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 1.3 mg of magnolol, and not less than 3.0 mg (for preparation prescribed 3.5 g of Rhubarb) or not less than 3.5 mg (for preparation prescribed 4 g of Rhubarb) of sennoside A ($C_{42}H_{38}O_{20}$: 862.74), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Hemp Fruit	5 g	4 g
Peony Root	2 g	2 g
Immature Orange	2 g	2 g
Magnolia Bark	2 g	2 g
Rhubarb	4 g	3.5 g
Apricot Kernel	2 g	2.5 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Mashiningan Extract occurs as a deep reddish yellow to very dark reddish yellow powder. It has a characteristic odor, and has a bitter taste and an astringent taste later.

Identification **(1)** Shake 0.5 g of Mashiningan Extract with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 0.3 g of powdered Hemp Fruit with 3 mL of methanol, 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 20 μL of the sample solution and 10 μL 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 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat the plate at 105℃ 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 to dark purple spot (*R*f value of about 0.5) from the standard solution (Hemp Fruit and Apricot Kernel).

(2) Shake 1.0 g of Mashiningan Extract with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin 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, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105℃ for 2 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot from the standard solution (Peony Root).

(3) Shake 1.0 g of Mashiningan Extract with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, shake 1.0 g of pulverized Immature Orange with 10 mL of methanol, 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 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, 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 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: two consecutive spots at *R*f values of about 0.7 obtained from the sample solution have respectively the same color tone and *R*f value with the blue-green spot and the blue spot immediately below it from the standard solution (Immature Orange).

(4) Shake 1.0 g of Mashiningan Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve 1 mg of magnolol 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 with fluorescent indicator 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. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Magnolia Bark).

(5) Shake 1.0 g of Mashiningan Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, 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, methanol and water (20:3:2) 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 orange fluorescent spot from the standard solution (Rhubarb).

(6) Shake 0.5 g of Mashiningan Extract with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of amygdalin 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, 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 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105℃ for 10 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 green to green-brown spot from the standard solution (Apricot Kernel).

Purity **(1)** Heavy metals *<1.07>*—Prepare the test solution with 1.0 g of Mashiningan Extract 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 Mashiningan Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying *<2.41>* Not more than 8.0% (1 g, 105℃, 5 hours).

Total ash *<5.01>* Not more than 9.0%.

Assay **(1)** Paeoniflorin—Weigh accurately about 0.5 g of Mashiningan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 30 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, then add 1 mL of acetic acid (100) and water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water *<2.48>* by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 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 paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T / A_S \times 5/8$$

M_S: Amount (mg) of Paeoniflorin RS taken, 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 μm in particle diameter).

Column temperature: A constant temperature of about 20℃.

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

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin 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 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(2) Magnolol—Weigh accurately about 0.5 g of Mashiningan Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 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 magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T / A_S \times 1/8$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 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℃.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol 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 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

(3) Sennoside A—Weigh accurately about 0.2 g of Mashiningan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 30 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, pour it into a column about 10 mm in inside diameter (previously prepared by packing 0.36 g of strongly basic ion-exchange resin for column chromatography, and washing with 10 mL of methanol and 10 mL of diluted methanol (1 in 2)) to elute, wash out the column with 10 mL of diluted methanol (1 in 2), then elute with a mixture of water, methanol and formic acid (25:25:1) to obtain exactly 5 mL of the effluent, and use this liquid as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water *<2.48>* by coulometric titration, using 10 mg), dissolve in 10 mL of diluted tetrahydrofuran (1 in 2), and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 25 mL of this solutions, add diluted methanol (1 in 2) to make exactly 100 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 sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T / A_S \times 1/16$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (2460:540:1).

Flow rate: 1.0 mL per minute.

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 sennoside A 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 sennoside A is not more than 1.5%.

Containers and storage Containers—Tight containers.