Shin'iseihaito Extract

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Shin'iseihaito Extract contains not less than 5 mg or not less than 20 mg of mangiferin, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 23 mg and not more than 69 mg (for preparation prescribed 1.5 g of Gardenia Fruit) or not less than 45 mg and not more than 135 mg (for preparation prescribed 3 g of Gardenia Fruit) of geniposide, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Magnolia Flower	3 g	2 g
Anemarrhena Rhizome	3 g	3 g
Lilium Bulb	3 g	3 g
Scutellaria Root	3 g	3 g
Gardenia Fruit	1.5 g	3 g
Ophiopogon Root	6 g	5 g
Gypsum	6 g	5 g
Cimicifuga Rhizome	1.5 g	1 g
Loquat Leaf	1 g	2 g

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

Description Shin'iseihaito Extract occurs as a reddish yellow to yellow-red powder. It has a slight odor, and a slightly bitter, slightly acid and slightly sweet taste.

Identification **(1)** To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then 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, to 1 g of powdered Magnolia Flower add 10 mL of methanol, shake, then 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 5 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 ethyl acetate and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105℃ for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark red-brown to brown spot (*R*f value: about 0.4) from the standard solution (Magnolia Flower).

(2) To 2.0 g of Shin'iseihaito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, to 1 g of pulverized Anemarrhena Rhizome add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL of the sample solution and 1 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-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105℃ for 2 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 yellowish red to dark red spot (*R*f value: about 0.3) from the standard solution (Anemarrhena Rhizome).

(3) To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), then add 2 mL of diethyl ether to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg of wogonin 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 20 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 hexane and acetone (7:5) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot from the standard solution (Scutellaria Root).

(4) To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of geniposide 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 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 7cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105℃ for 1 minute: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to dark purple spot from the standard solution (Gardenia Fruit).

(5) Place 2.0 g of Shin'iseihaito Extract in a porcelain crucible, and ignite to incinerate at 500 – 550℃. To the residue add 60 mL of water, shake, then centrifuge, and use the supernatant liquid as the sample solution. Add ammonium

oxalate TS to the sample solution: a white precipitate is formed. The precipitate does not dissolve by the addition of diluted acetic acid, but dissolves by the addition of diluted hydrochloric acid (Gypsum).

(6) To 1.0 g of Shin'iseihaito add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Use (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography 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, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat the plate at 105℃ for 5 minutes, and 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 light yellow-white to yellow-green fluorescent spot from the standard solution (Cimicifuga Rhizome).

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

Loss on drying *<2.41>* Not more than 9.0% (1 g, 105℃, 5 hours).

Total ash *<5.01>* Not more than 14.0%.

Assay **(1)** Mangiferin—Weigh accurately about 0.5 g of Shin'iseihaito, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, then centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of mangiferin for assay, dissolve in diluted methanol (1 in 2) 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 mangiferin in each solution.

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

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength:367 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 phosphoric acid (1780:220:1).

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 mangiferin 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 mangiferin is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of Shin'iseihaito, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, then filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water *<2.48>* by coulometric titration, using 10 mg), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly10 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 baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T / A_S \times 1/4$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 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 diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

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 baicalin 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 baicalin is not more than 1.5%.

(3) Geniposide — Weigh accurately about 0.5 g of Shin'iseihaito, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, then centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in 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 geniposide in each solution.

Amount (mg) of geniposide $= M_S \times A_T / A_S \times 1/2$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 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 (900:100:1).

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 geniposide 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 geniposide is not more than 1.5%.

Containers and storage Containers — Tight containers.