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Saireito Extract

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Saireito Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.37), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 7 g of Bupleurum Root, 5 g of Pinellia Tuber, 1 g of Ginger, 3 g of Scutellaria Root, 3 g of Jujube, 3 g of Ginseng, 2 g of Glycyrrhiza, 6 g of Alisma Rhizome, 4.5 g of Polyporus Sclerotium, 4.5 g of Poria Sclerotium, 4.5 g of Atractylodes Rhizome and 3 g of Cinnamon Bark, or with 7 g of Bupleurum Root, 5 g of Pinellia Tuber, 1 g of Ginger, 3 g of Scutellaria Root, 3 g of Jujube, 3 g of Ginseng, 2 g of Glycyrrhiza, 5 g of Alisma Rhizome, 3 g of Polyporus Sclerotium, 3 g of Poria Sclerotium, 3 g of Atractylodes Lancea Rhizome and 2 g of Cinnamon Bark.

Description Saireito Extract occurs as a light yellow-brown powder. It has slightly a characteristic odor, and a sweet, then bitter taste.

Identification **(1)** Bupleurum root—To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 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, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution.

(2) Ginger—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether layer

under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 15 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 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 from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution.

(3) Scutellaria root—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this 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 ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution.

(4) Ginseng—To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 Reference Standard 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 from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution.

(5) Glycyrrhiza—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution.

(6) Alisma rhizome—To 2.0 g of Saireito Extract add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A 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 40 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, hexane and acetic acid (100) (10:10:3) 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 from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution.

(7) Atractylodes rhizome (for preparation prescribed Atractylodes Rhizome)—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plat, heat at 105°C for 5 minutes, examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(8) Atractylodes lancea rhizome (for preparation prescribed Atractylodes Lancea Rhizome)—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around *R*f 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool.

(9) Cinnamon bark—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid 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 40 μL of the sample solution and 2 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) 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 from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Saireito Extract as directed in (4) in Extracts under the General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Saireito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> Not more than 10.0% (1 g, 105°C, 5 hours).

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

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b_2 for component determination, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, 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 saikosaponin b_2.

Amount (mg) of saikosaponin $b_2 = W_S \times (A_T/A_S) \times (1/20)$

W_S: Amount (mg) of saikosaponin b_2 for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3)

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b_2 is about 12 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 saikosaponin b_2 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 saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of Saireito Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (separately determine the water), dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform test with exactly 10 μL each of the sample solution and the 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.

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

W_S: Amount (mg) of Baicalin Reference Standard, 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°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6)

Flow rate: 1.0 mL/min. (the retention time of baicalin is about 10 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 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) Glycyrrhizic acid—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), 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 the 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 glycyrrhizic acid.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= W_S \times (A_T/A_S) \times (1/2)$

W_S: Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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).

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Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 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 glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

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