JP XVIII (2021)

Otsujito Extract

乙字湯エキス

Otsujito Extract contains not less than 1.2 mg and not more than 4.8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 14 mg and not more than 42 mg (for preparation prescribed 2 g of Glycyrrhiza) or not less than 20 mg and not more than 60 mg (for preparation prescribed 3 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 0.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 1.5 mg of rhein (for preparation prescribed 0.5 g of Rhubarb) or not less than 1 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 3 mg of rhein (for preparation prescribed 1 g of Rhubarb), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Japanese Angelica Root	6 g	6 g	6 g
Bupleurum Root	5 g	5 g	5 g
Scutellaria Root	3 g	3 g	3 g
Glycyrrhiza	2 g	2 g	3 g
Cimicifuga Rhizome	1.5 g	1 g	1 g
Rhubarb	1 g	0.5 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above.

Description Otsujito Extract occurs as light brown to brown powder or black-brown viscous extract, having a slightly order, and a hot and slight sweet taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, and use the diethyl ether layer as the sample solution. Separately, use (*Z*)-ligustilide 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 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 butyl acetate and hexane (2:1) 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 blue-white fluorescent spot from the standard solution (Japanese Angelica Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous 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 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 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105°C for 5 minutes. 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 yellow fluorescent spot from the standard solution (Bupleurum Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, 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, 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 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, hexane and acetic acid (100) (10:10:1) 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) Shake 1.0 g of the dry extract (or 3.0 g of the viscous 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 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 1 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, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C 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 yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, 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°C 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 fluorescent spot from the standard solution (Cimicifuga Rhizome).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, 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, 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).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.5%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, shake for 10 minutes, and centrifuge. After removing the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately use saikosaponin b_2 standard TS for assay 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 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

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 per minute (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 the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), 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 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 exactly 10 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°C.

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

Flow rate: 1.0 mL per minute (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 the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, shake for 10 minutes, and centrifuge. After removing the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 glycyrrhizic acid in each solution.

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

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, 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).

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

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

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 glycyrrhizic acid is not more than 1.5%.

(4) Sennoside A—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 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 flow out, wash out the column with 10 mL of diluted methanol (1 in 2), then flow out with a mixture of water, methanol and formic acid (25:25:1) to obtain exactly 5 mL of the outflow liquid, and use this liquid as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/8$$

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 (the retention time of sennoside A is about 14 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 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%.

(5) Rhein—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 80 mL of water, shake, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 20 mL of iron (III) chloride TS, heat under a reflux condenser for 30 minutes, add 3 mL of hydrochloric acid, and heat in addition under a reflux condenser for 30 minutes. After cooling, extract three times with 25 mL each of diethyl ether, combine all the diethyl ether layers, evaporate the solvent under low pressure (in vacuo), dissolve the residue in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhein for assay, and dissolve in acetone 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 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 rhein in each solution.

$$\text{Amount (mg) of rhein} = M_S \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of rhein for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of rhein is about 17 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 rhein 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 rhein is not more than 1.5%.

Containers and storage Containers—Tight containers.