Saikokeishikankyoto Extract

柴胡桂枝乾姜湯エキス

Saikokeishikankyoto Extract contains not less than 1.4 mg and not more than 5.6 mg of saikosaponin b_2, not less than 78 mg and not more than 234 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 15 mg and not more than 45 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Bupleurum Root	6 g	6 g
Cinnamon Bark	3 g	3 g
Scutellaria Root	3 g	3 g
Oyster Shell	3 g	3 g
Processed Ginger	2 g	3 g
Glycyrrhiza	2 g	2 g
Trichosanthes Root	3 g	4 g

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

Description The dry extract occurs as a light yellow-brown to brown powder. It has a characteristic odor and a hot, bitter and slightly sweet taste.

The viscous extract occurs as a black-brown viscous liquid. It has a characteristic odor and a bitter, hot and slightly sweet taste, followed by an astringent aftertaste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, 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 5 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).

(2) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of dry extract (or 30 g of the viscous extract) in a 300-mL of hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde 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, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine 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-orange to orange spot from the standard solution.

ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, add 5 mL of hexane, shake, centrifuge, and use the hexane layer as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde 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 ethyl acetate (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.

(3) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under a low pressure (vacuum), 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 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 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 the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under a low pressure (vacuum), add 2 mL of diethyl ether to the residue, and use this solution 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 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 and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4- dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Processed Ginger).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, 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 to yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

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 the dried substance) 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 the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the 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 13.0%, 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, and shake for 10 minutes. Centrifuge, remove 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 water 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, and 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.

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), and 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.

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—Perform the test according to the following i) or ii).

i) 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, filter, and use the filtrate 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 exactly100 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.

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%. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography and 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, two peaks other than glycyrrhizic acid are observed with the resolutions between the peak of glycyrrhizic acid and each of the two peaks being 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%.

ii) 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, and shake for 10 minutes. Centrifuge, remove 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 water 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, and 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, 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 exactly100 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.

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—
Proceed as directed in the operating conditions in i).
System suitability—
System repeatability: Proceed as directed in the system suitability in i).
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.

Containers and storage Containers—Tight containers.