

# JP XVIII

## (2021)

### Daiokanzoto Extract

大黄甘草湯エキス

Daiokanzoto Extract contains not less than 3.5 mg of sennoside A ( $C_{42}H_{38}O_{20}$ : 862.74), and not less than 7 mg and not more than 21 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 14 mg and not more than 42 mg (for preparation prescribed 2 g of Glycyrrhiza) 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)
Rhubarb	4 g	4 g
Glycyrrhiza	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** Daiokanzoto Extract occurs as a brown powder. It has a characteristic odor and an astringent first then slightly sweet taste.

**Identification (1)** To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then 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 5  $\mu$ L 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. 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).

**(2)** To 0.5 g of Daiokanzoto 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 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  $\mu$ L 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).

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

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

**Loss on drying <2.41>** Not more than 7.0% (1 g, 105°C, 5 hours).

**Total ash <5.01>** Not more than 10.0%.

**Assay (1)** Sennoside A—Weigh accurately about 0.2 g of Daiokanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, and remove the ethyl acetate layer. To the aqueous layer add 20 mL of ethyl acetate, shake for 10 minutes, centrifuge, and remove the ethyl acetate 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 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  $\mu$ L 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.

$$\begin{aligned} & \text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) \\ & = M_S \times A_T / A_S \times 1/4 \end{aligned}$$

$M_S$ : 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  $\mu$ m 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  $\mu$ L 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  $\mu$ L 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%.

**(2)** Glycyrrhizic acid—Weigh accurately about 0.2 g of

Daikanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the ethyl acetate layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the ethyl acetate 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  $\mu$ L 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.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ & = M_S \times A_T / A_S \times 1/2 \end{aligned}$$

$M_S$ : 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  $\mu$ m 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  $\mu$ L 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  $\mu$ L 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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