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Daiokanzoto Extract

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Daiokanzoto Extract contains not less than 3.5 mg and not more than 10.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) and not less than 9 mg and not more than 27 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 18 mg and not more than 54 mg (for preparation prescribed 2 g of Glycyrrhiza) 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 4 g of Rhubarb and 1 g of Glycyrrhiza, or with 4 g of Rhubarb and 2 g of Glycyrrhiza.

Description Daiokanzoto Extract occurs as a brown powder. It has a characteristic odor and an astringent first then slightly sweet taste.

Identification **(1)** Rhubarb—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 supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 1 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 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 10 cm, and air-dry the plate. 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 orange fluorescent spot from the standard solution.

(2) Glycyrrhiza—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 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 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, 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.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Daiokanzoto 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 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 upper layer. To the water layer add 20 mL of ethyl acetate, shake for 10 minutes, centrifuge, and remove the upper layer. To the water 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 Reference Standard (separately determine the water), 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.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = W_S \times (A_T/A_S) \times (1/4)$$

W_S: Amount (mg) of Sennoside A Reference Standard, 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/min (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%.

(2) Glycyrrhizic acid—Use the sample solution obtained in the Assay (1) 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 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.

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

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).

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: 1.0mL/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.