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## Shakuyakukanzoto Extract

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Shakuyakukanzoto Extract contains not less than 50 mg and not more than 150 mg of paeoniflorin ( $C_{23}H_{28}O_{11}$ : 480.46), and not less than 40 mg and not more than 120 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)
Peony Root	6 g	5 g
Glycyrrhiza	6 g	5 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** Shakuyakukanzoto Extract occurs as a light brown powder or brown viscous extract. It has slightly an odor, and a sweet taste.

**Identification (1)** Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin 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  $\mu$ 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 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R<sub>f</sub>* value with the purple spot from the standard solution (Peony Root).

(2) Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of water, 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 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<sub>f</sub>* 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 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 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <2.41> The dry extract: Not more than 8.0% (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 9.0%, calculated on the dried basis.

**Assay** (1) Paeoniflorin—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to 0.2 g of 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 Paeoniflorin 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<sub>T</sub>* and *A<sub>S</sub>*, of paeoniflorin in each solution.

$$\begin{aligned} &\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}\text{)} \\ &= M_S \times A_T/A_S \times 1/2 \end{aligned}$$

*M<sub>S</sub>*: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

**Operating conditions**—

Detector: An ultraviolet absorption photometer (wavelength: 232 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

20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

**System suitability**—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

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 paeoniflorin is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.2 g of 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 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<sub>T</sub>* and *A<sub>S</sub>*, of glycyrrhizic acid in each solution.

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

*M<sub>S</sub>*: 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%.

**Containers and storage** Containers—Tight containers.