

Otsujito Extract

乙字湯エキス

Change the Origin/limits of content, Identification (4) and Assay (3) as follows:

Otsujito Extract contains not less than 1.2 mg and not more than 4.8 mg of saikosaponin b₂, not less than 80 mg and not more than 240 mg of baicalin (C₂₁H₁₈O₁₁: 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 (C₄₂H₆₂O₁₆: 822.93), and not less than 0.5 mg of sennoside A (C₄₂H₃₈O₂₀: 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 (C₄₂H₃₈O₂₀: 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.

Identification

(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 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 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).

Assay

(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, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant 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.

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

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 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%.

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