Bofutsushosan Extract

防風通聖散エキス

Change the Origin/limits of content as follows:

Bofutsushosan Extract contains not less than 9 mg and not more than 36 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 4 mg and not more than 12 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 54 mg and not more than 162 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 13 mg and not more than 39 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Change the Identification (7), (8), (9) and (15) as follows:

Identification

(7) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of 0.1 mol/L hydrochloric acid TS, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid 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, water and acetic acid (100) (60:1:1) to a distance of about 10 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 greenish brown spot from the standard solution (Schizonepeta Spike; Mentha Herb).

(8) For preparation prescribed Saposhnikovia Root and Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glycosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this so-

lution 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 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, 1-propanol, water and acetic acid (100) (7:5:4:1) 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 2 minutes, then 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 (Saposhnikovia Root and Rhizome).

(9) For preparation prescribed Glehnia Root and Rhizome—To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 5 mL of ethyl acetate, and heat on a water bath under a reflux condenser for 30 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of scopoletin for thin-layer chromatography in 10 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 ethyl acetate and hexane (3:1) 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 blue-white fluorescent spot from the standard solution (Glehnia Root and Rhizome).

(15) To 1.0 g of the dry extract (or 3.0 g of the viscous 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 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).

Add the following next to the Identification (17) as follows:

Identification

(18) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a crucible, and ignite at 550°C for 5 hours to incinerate. To the residue add 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. After cooling, add 20 mL of water, shake, and filter. To 5 mL of the filtrate add ammonia TS until a white gelatinous precipitate is formed, centrifuge, and remove the supernatant liquid. To the residue add 5 mL of water, shake, centrifuge, and remove the supernatant liquid. Then, to the residue add 5 mL of water, shake, centrifuge, and remove the supernatant liquid. To the obtained residue add 5 drops of alizarin red S TS, and shake occasionally in lukewarm water: the residue is red to red-brown in color (Kasseki).

Change the Assay (4) as follows:

Assay

(4) 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 ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, 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.

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

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 (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 solu-

tion 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%.