

Bofutsushosan Extract

防風通聖散エキス

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 and pseudoephedrine), 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.

Method of preparation

	1)	2)	3)	4)	5)	6)
Japanese Angelica						
Root	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Peony Root	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Cnidium Rhizome	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Gardenia Fruit	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Forsythia Fruit	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Mentha Herb	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Ginger	0.3 g	0.3 g	0.4 g	0.4 g	1.2 g	0.3 g
Schizonepeta Spike	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Saposhnikovia Root and Rhizome	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	—
Glehnia Root and Rhizome	—	—	—	—	—	1.2 g
Ephedra Herb	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Rhubarb	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Sodium Sulfate	—	1.5 g	—	1.5 g	—	—
Anhydrous Sodium Sulfate	0.7 g	—	0.75 g	—	1.5 g	0.75 g
Attractylodes						
Rhizome	2 g	2 g	2 g	2 g	2 g	2 g
Platycodon Root	2 g	2 g	2 g	2 g	2 g	2 g
Scutellaria Root	2 g	2 g	2 g	2 g	2 g	2 g
Glycyrrhiza	2 g	2 g	2 g	2 g	2 g	2 g
Gypsum	2 g	2 g	2 g	2 g	2 g	2 g
Aluminum Silicate Hydrate with Silicon Dioxide	3 g	3 g	3 g	3 g	3 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description Bofutsushosan Extract is a yellow-brown to brown powder or black-brown viscous extract. It has a slightly odor and a sweet and slightly bitter taste.

Identification (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, and use the diethyl ether layer as the sample solution. Separately, use (*Z*)-ligustilide TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution and 10 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) 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 blue-white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) 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 1-butanol layer as the sample solution. Separately, dissolve 1 mg of 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 10 μ L of the sample solution and 5 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 1 minute: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-purple to purple spot from the standard solution (Peony Root).

(3) 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 1-butanol layer as the sample solution. Separately, dissolve 1 mg of geniposide 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 10 μ L of the sample solution and 5 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) 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 1 minute: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-purple to purple spot from the standard solution (Gardenia Fruit).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, to 1.0 g of pulverized forsythia fruit add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution and 10 μ L of the standard solution as bands on the original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (10:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-purple spot (*R_f* value: about 0.4) from the standard solution (Forsythia Fruit).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the ethyl acetate layer as the sample solution. Separately, shake 0.2 g of pulverized mentha herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, shake, centrifuge, and use the ethyl acetate layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ 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 acetone, ethyl acetate, water, and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, heat the plate at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-brown spot (*R_f* value: around 0.4) from the standard solution (Mentha Herb).

(6) Perform the test according to the following (i) or (ii) (Ginger).

(i) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 20 μ L of the sample solution and 5 μ L 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 (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the blue-green to grayish green spot from the standard solution.

(ii) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol 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 20 μ L of the sample solution and 5 μ L 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 (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the blue-green to grayish green spot from the standard solution.

(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, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), 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 μ 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, 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 1-butanol layer 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 solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution and 5 μ L 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 *Rf* 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 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 μ L of the sample solution and 2 μ L 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 *Rf* value with the blue-white fluorescent spot from the standard solution (Glehnia Root and Rhizome).

(10) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the diethyl ether layer as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 15 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat the plate at 105°C for 5 minutes: a red-purple spot is observed at about 0.5 of *Rf* value (Ephedra Herb).

(11) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use this solution 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 10 μ L of the sample solution and 5 μ L of the 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 spot among the several spots obtained from the sample solution

has the same color tone and *Rf* value with the orange fluorescent spot from the standard solution (Rhubarb).

(12) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), then dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 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 μ L of the sample solution and 5 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *Rf* value with the red to red-purple spot from the standard solution (Atractyloides Rhizome).

(13) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium carbonate TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, to 2.0 g of pulverized platycodon root add 10 mL of sodium carbonate TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ 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 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat the plate at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *Rf* value with the blue-purple spot (*Rf* value: about 0.4) from the standard solution (Platycodon Root).

(14) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, centrifuge, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of wogonin 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 20 μ L of the sample solution and 2 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *Rf* value with the yellow-brown to grayish brown spot from the standard solution (Scutellaria Root).

(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 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 μ L each of the sample solution and stand-

ard 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).

(16) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a porcelain crucible, ignite to incinerate at 550°C, then to the residue add 60 mL of water, shake, centrifuge, and use the supernatant as the sample solution. Add ammonium oxalate TS to the sample solution: a white precipitate is formed. The precipitate does not dissolve in diluted acetic acid, but dissolve on the addition of diluted hydrochloric acid (Gypsum).

(17) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a porcelain crucible, ignite to incinerate at 550°C. To the residue add 60 mL of water, shake well, centrifuge, and use the supernatant as the sample solution. The sample solution responds to the Qualitative Tests <1.09> (1) for sulfate (Gypsum; Sodium Sulfate or Anhydrous Sodium Sulfate).

(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 shows red to red-brown in color (Kasseki).

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 the 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 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 9.0% (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 less than 10.0% and more than 22.0%, calculated on the dried basis.

Assay (1) Paeoniflorin—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 exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, elute through a column packed with 2 g of polyamide for column chromatography using 20 mL of water, then add 1 mL of acetic acid (100), add water to make exactly 25 mL, and use this solution 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), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make

exactly 20 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 paeoniflorin in each solution.

$$\begin{aligned} &\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ &= M_S \times A_T / A_S \times 5/8 \end{aligned}$$

M_S: 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) Total alkaloids (ephedrine and pseudoephedrine)—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, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as above, and remove the diethyl ether layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the diethyl ether layer. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the extracts, evaporate the solvent under low pressure (in vacuo), dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drug, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 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. Determine the peak areas, *A_{TE}* and *A_{TP}*, of ephedrine and pseudoephedrine obtained with the sample solution, and the peak area, *A_S*, of ephedrine obtained with the standard solution.

Amount (mg) of total alkaloids (ephedrine and pseudoephedrine)

$$= M_S \times (A_{TE} + A_{TP}) / A_S \times 1/10 \times 0.819$$

M_S : Amount (mg) of ephedrine hydrochloride for assay of crude drug taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drug and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(3) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ 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 baicalin in each solution.

$$\begin{aligned} &\text{Amount (mg) of baicalin (C}_{21}\text{H}_{18}\text{O}_{11}) \\ &= M_S \times A_T/A_S \times 1/4 \end{aligned}$$

M_S : Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 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 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times

with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(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 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 μ 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 μ 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 μ 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 μ 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.