

Add the following:**Maoto Extract**

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Maoto Extract contains not less than 15 mg and not more than 45 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 48 mg and not more than 192 mg of amygdalin, and not less than 14 mg and not more than 42 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)
Ephedra Herb	5 g
Apricot Kernel	5 g
Cinnamon Bark	4 g
Glycyrrhiza	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, or prepare a dry extract by adding Light Anhydrous Silicic Acid to the extractive prepared as directed under Extracts, according to the prescription 1), using the crude drugs shown above.

Description Maoto Extract occurs as light brown to blackish brown, powder or viscous extract, having a slightly order, and a sweet and bitter, then a slightly astringent taste.

Identification (1) 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. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 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 at 105°C for 5 minutes: a red-purple spot is observed at an *R_f* value of about 0.5 (Ephedra Herb).

(2) 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 2 mg of amygdalin 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 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 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 green-brown spot obtained from the standard solution (Apricot Kernel).

(3) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde 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 40 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 hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde 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 40 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 hexane and ethyl acetate (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 spot among the several spots obtained from the sample solution has the same color tone and *R_f* value with the bluish white fluorescent spot obtained from the standard solution.

(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 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, 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, and heat 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 yellow-brown spot obtained 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 in 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 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 9.5% (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 13.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 10.0% and 22.0%.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 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 upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper 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 supernatant liquid. 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 supernatant liquids, evaporate the solvent under reduced pressure, 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 drugs, 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 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_{TE} and A_{TP} , of ephedrine and pseudoephedrine obtained from the sample solution, and peak area, A_S , of ephedrine from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
 $= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter 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 to dissolve lauryl sulfate.

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 drugs 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%.

(2) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 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, flow through in a column packed with 2 g of polyamide for column chromatography, then elute with water to make exactly 20 mL, and use this effluent as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in diluted methanol (1 in 2) to make exactly 50 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 amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S : Amount (mg) of amygdalin for assay

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 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin 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 amygdalin is not more than 1.5%.

(3) Glycyrrhizinic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the 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 Glycyrrhizinic Acid RS (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 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 glycyrrhizinic acid in each solution.

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

M_S : Amount (mg) of Glycyrrhizinic Acid RS, 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: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizinic acid is about 12 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 glycyrrhizinic acid 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 glycyrrhizinic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

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