Unseiin Extract

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Unseiin Extract contains not less than 24 mg and not more than 72 mg (for preparation prescribed 3 g of Peony Root), or not less than 32 mg and not more than 96 mg (for preparation prescribed 4 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$:480.46), not less than 39 mg and not more than 117 mg (for preparation prescribed 1.5 g of Scutellaria Root), or not less than 78 mg and not more than 234 mg (for preparation prescribed 3 g of Scutellaria Root) of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 20 mg and not more than 60 mg of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Japanese Angelica Root	4 g	4 g	3 g
Rehmannia Root	4 g	4 g	3 g
Peony Root	3 g	4 g	3 g
Cnidium Rhizome	3 g	4 g	3 g
Scutellaria Root	3 g	3 g	1.5 g
Gardenia Fruit	2 g	2 g	1.5 g
Coptis Rhizome	1.5 g	1.5 g	1.5 g
Phellodendron Bark	1.5 g	1.5 g	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above.

Description Unseiin Extract occurs as a yellow-brown to very dark brown powder or blackish brown viscous extract. It has a slight odor, and has a slightly sweet taste at first, followed by a pungent taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution 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 10 μL each of the sample solution 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 bluish-white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid 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 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 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105℃ for 1 minute: one of 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 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then add 2 mL of diethyl ether to the residue, and use this 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 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, 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 several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot from the standard solution (Scutellaria Root).

(4) To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid 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 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, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, and heat at 105℃ for 1 minute: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the purple to dark purple spot from the standard solution (Gardenia Fruit).

(5) To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of coptisine chloride for thin-layer chromatography in 5 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 2 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, ammonia solution (28) and methanol (15:1:1) 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 yellow fluorescent spot from the standard solution (Coptis Rhizome).

(6) To 1.0 g of dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of limonin 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 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, hexane and acetic acid (100) (10:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat at 105℃ for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the purple to dark purple spot from the standard solution (Phellodendron Bark).

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 10.0% (1 g, 105℃, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105℃, 5 hours).

Total ash *<5.01>* Not more than 9.0%, 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, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, then add 1 mL of acetic acid (100) to the eluate, 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.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T / A_S \times 5/8$$

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℃.

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

Flow rate: 1.0 mL per minute.

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) 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, then 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), and 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 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 baicalin in each solution.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$)
$= M_S \times A_T / A_S \times 1/4$

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 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

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

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL 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 μL 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%.

(3) Berberine—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 the mobile phase, shake for 15 minutes, then filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase 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 berberine in each solution.

Amount (mg) of berberine chloride ($C_{20}H_{18}ClNO_4$)
$= M_S \times A_T / A_S \times 1/2$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 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 30℃.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatine and berberine 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 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.