

Add the following:

Hachimijiogan Extract

八味地黄丸エキス

Hachimijiogan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg (for preparation prescribed 3 g of Moutan Bark) or not less than 5 mg and not more than 15 mg (for preparation prescribed 2.5 g of Moutan Bark) of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride), or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) per the extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 5 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Poria Sclerotium, 3 g of Moutan Bark, 1 g of Cinnamon Bark, and 1 g of Processed Aconite Root 1 of “Processed Aconite Root” or Powdered Processed Aconite Root 1 or Powdered Processed Aconite Root 2 of “Powdered Processed Aconite Root”, or with 6 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Poria Sclerotium, 2.5 g of Moutan Bark, 1 g of Cinnamon Bark, and 0.5 g of Powdered Processed Aconite Root 1 of “Powdered Processed Aconite Root”.

Description Hachimijiogan Extract occurs as grayish brown to black-brown powder or viscous extract. It has a characteristic odor and a slightly bitter and acid taste.

Identification (1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with these solutions 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 water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at around R_f 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol,

shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-layer Chromatography <2.03>. Spot 10 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, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Cornus Fruit).

(3) 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 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Alisol A for thin-layer chromatography in 1 mL of methanol, and use the 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) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Alisma Rhizome).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use the 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 hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the orange spot from the standard solution (Moutan Bark).

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

(i) Put 10 g of the dry extract (or 30 g of the 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 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid 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 50 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 hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, 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 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 hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among several spots from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylemesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), 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 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

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 in the Extracts (4) under General Rules for Preparations, 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).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hyaconitine and mesaconitine)—Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to

1.0 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. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet exactly 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hyaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hyaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hyaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5 %.

Loss on drying <2.41> The dry extract: Not more than 8.5 % (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 10.0%, calculated on the dried basis.

Assay (1) Loganin—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, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of loganin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and 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 loganin.

$$\text{Amount (mg) of loganin} = W_S \times (A_T / A_S) \times (1/2)$$

W_S : Amount (mg) of loganin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 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 symmetry factor of the peak of loganin 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 loganin is not more than 1.5%.

(2) Peoniflorin—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, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water), and 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 peoniflorin.

$$\begin{aligned} \text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ = W_S \times (A_T / A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Peoniflorin Reference Standard, 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 octadecylsilylated silica gel for liquid chromatography (5 μ m 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 peoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin Reference Standard and albiflorin 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, albiflorin and peoniflorin 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 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 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. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μ L each of the sample solution and the aconitum monoester alkaloids standard solution TS for component determination as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM} , A_{TH} and A_{SH} , as well as A_{TA} and A_{SA} , in each solution, respectively.

$$\begin{aligned} \text{Amount (mg) of benzoylmesaconine hydrochloride} \\ = C_{SM} \times (A_{TM} / A_{SM}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of benzoylhypaconine hydrochloride} \\ = C_{SH} \times (A_{TH} / A_{SH}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of 14-anisoylaconine hydrochloride} \\ = C_{SA} \times (A_{TA} / A_{SA}) \times 10 \end{aligned}$$

C_{SM} : Concentration (mg/mL) of benzoylmesaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SH} : Concentration (mg/mL) of benzoylhypaconine hydrochloride for component determination in aconitum

monoester alkaloids standard solution TS for component determination

C_{SA} : Concentration (mg/mL) of 14-anisoylaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylhypaconine and benzoylmesaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μ L of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Container and storage Containers—Tight containers.

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