

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

Change to read:

Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 48 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikosaponin b₂, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 0.5 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 3 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Processed Ginger and 0.5 g of Cimicifuga Rhizome.

Description Hochuekkito Extract occurs as a light brown to black-brown powder or viscous extract. It has a slight odor, and a sweet and bitter taste.

Identification (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and shake. Take the 1-butanol layer, evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb₁ Reference Standard 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, 1-propanol, water and acetic acid (100) (7:5:4:1) 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 (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III 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 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 ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-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 red spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, shake, and take the hexane layer. To the hexane layer add anhydrous sodium sulfate to dry, filter, evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot appears around R_f 0.4, which shows a greenish brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allowing to cool (Atractylodes Lancea Rhizome).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 40 mL of a solution of potassium hydroxide in methanol (1 in 50), shake for 15 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 30 mL of water to the residue, then add 20 mL of diethyl ether, shake, and take the water layer. To the water layer add 20 mL of 1-butanol, shake, and take the 1-butanol layer. To the 1-butanol layer add 20 mL of water, shake, take the 1-butanol layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside IV 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 octadecylsilylized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water, 1-butanol and acetic acid (100) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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 red-brown spot from the standard solution (Astragalus Root).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide 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 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chro-

matography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) 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 the 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 (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin 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 2 μ L of the sample solution and 20 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, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue spot from the standard solution (Citrus Unshiu Peel).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin *b*₂ 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 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, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde 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 red spot from the standard solution (Bupleurum Root).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution 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 10 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 from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(9) For preparation prescribed Ginger—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this 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 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Ginger).

(10) For preparation prescribed Processed Ginger—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 an 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 about 1 hour, separate the hexane layer, and use this 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 60 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 cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Processed Ginger).

(11) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 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, acetone and water

(20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow fluorescent spot from the standard solution (Cimicifuga Rhizome).

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).

Loss on drying <2.41> The dry extract: Not more than 11.5% (1 g, 105°C, 5 hours).

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

Total ash <5.01> Not more than 9.0%, calculated on the dried basis.

Assay (1) Hesperidin—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 tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) 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 hesperidin.

$$\text{Amount (mg) of hesperidin} = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of hesperidin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL/min. (the retention time of hesperidin is about 15 minutes.)

System suitability—

System performance: Dissolve 1 mg each of hesperidin for component determination and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin 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 hesperidin is not more than 1.5%.

(2) Saikosaponin b₂—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 saikosaponin b₂ 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. Pipet 10 mL of this solution, add 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 saikosaponin b₂.

$$\text{Amount (mg) of saikosaponin } b_2 = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of saikosaponin b₂ for component determination

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: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b₂ is about 12 minutes.)

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 saikosaponin b₂ 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 saikosaponin b₂ is not more than 1.5%.

(3) 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 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 Glycyrrhizic Acid Reference Standard (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 glycyrrhizic acid.

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

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, 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/min. (the retention time of glycyrrhizic 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 glycyrrhizic 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*

*Suppl I,
JP XV
(2007)*