

# JP XVIII

## (2021)

### Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 64 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 b<sub>2</sub>, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid (C<sub>42</sub>H<sub>62</sub>O<sub>16</sub>: 822.93), per extract prepared with the amount specified in the Method of preparation.

#### Method of preparation

	1)	2)	3)	4)	5)	6)
Ginseng	4 g	4 g	4 g	4 g	4 g	4 g
Atractylodes Rhizome	4 g	—	4 g	—	4 g	4 g
Atractylodes Lancea Rhizom	—	4 g	—	4 g	—	—
Astragalus Root	4 g	4 g	4 g	4 g	3 g	4 g
Japanese Angelica Root	3 g	3 g	3 g	3 g	3 g	3 g
Citrus Unshiu Peel	2 g	2 g	2 g	2 g	2 g	2 g
Jujube	2 g	2 g	2 g	2 g	2 g	2 g
Bupleurum Root	2 g	2 g	1 g	1 g	2 g	1 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Ginger	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	—
Processed Ginger	—	—	—	—	—	0.5 g
Cimicifuga Rhizome	1 g	1 g	0.5 g	0.5 g	1 g	0.5 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** Hochuekkito Extract occurs as a light brown to brown powder or black-brown 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 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb<sub>1</sub> RS or ginsenoside Rb<sub>1</sub> 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, 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 vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and R<sub>f</sub> value with the blue-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 separate the diethyl ether layer. Evaporate the solvent under low pressure (in vacuo), 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 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 ethyl acetate and hexane (1:1) 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 several spots obtained from the sample solution has the same color tone and R<sub>f</sub> 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 separate the hexane layer. Evaporate the solvent under low pressure (in vacuo), 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 μL 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an R<sub>f</sub> value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated the plate at 105°C for 5 minutes, and allowed 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 solvent under low pressure (in vacuo). Add 30 mL of water and 20 mL of diethyl ether to the residue, shake, remove the diethyl ether layer, and separate the aqueous layer. To the aqueous layer add 20 mL of 1-butanol, shake, and separate the 1-butanol layer. To the 1-butanol

layer add 20 mL of water, shake, separate the 1-butanol layer, evaporate the solvent under low pressure (in vacuo), 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  $\mu\text{L}$  each of the sample solution and standard solution on a plate of octadecylsilanized 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 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained 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 separate the diethyl ether layer. Evaporate the solvent under low pressure (in vacuo), add 1 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  $\mu\text{L}$  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 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).

(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 separate the 1-butanol layer. Evaporate the solvent under low pressure (in vacuo), 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  $\mu\text{L}$  of the sample solution and 20  $\mu\text{L}$  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 7 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 several spots obtained 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 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, and shake. Centrifuge this solution, and use the 1-butanol layer 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  $\mu\text{L}$  of the sample solution and 2  $\mu\text{L}$  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 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,

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 fluorescent 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, shake, and separate the 1-butanol layer. Evaporate the solvent under low pressure (in vacuo), 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 1  $\mu\text{L}$  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, 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).

(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 separate the diethyl ether layer. Evaporate the solvent under low pressure (in vacuo), 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  $\mu\text{L}$  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 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 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 (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 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 [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  $\mu\text{L}$  of the sample solution and 10  $\mu\text{L}$  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 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 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 (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, shake, and separate the 1-butanol layer. Evaporate the solvent under low pressure (in vacuo), add 3 mL of methanol to the residue, and use this solution as the sample solution. Use (*E*)-isoferulic 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  $\mu$ L of the sample solution and 2  $\mu$ 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 7 cm, and air-dry the plate. Spray evenly 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<sub>f</sub>* value with the light yellow-white 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), 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 assay, 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  $\mu$ 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<sub>T</sub>* and *A<sub>S</sub>*, of hesperidin in each solution.

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

*M<sub>S</sub>*: Amount (mg) of hesperidin for assay taken

**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  $\mu$ m 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 per minute (the retention time of hesperidin is about 15 minutes).

**System suitability**—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10  $\mu$ L 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  $\mu$ L 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 *b*<sub>2</sub>—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, use saikosaponin *b*<sub>2</sub> standard TS for assay as the standard solution. Perform the test with exactly 10  $\mu$ 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<sub>T</sub>* and *A<sub>S</sub>*, of saikosaponin *b*<sub>2</sub> in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

*C<sub>S</sub>*: Concentration (mg/mL) of saikosaponin *b*<sub>2</sub> in saikosaponin *b*<sub>2</sub> standard TS for assay

**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  $\mu$ m 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 per minute (the retention time of saikosaponin *b*<sub>2</sub> is about 12 minutes).

**System suitability**—

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin *b*<sub>2</sub> are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin *b*<sub>2</sub> 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 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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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.

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