# Hangekobokuto Extract

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Hangekobokuto Extract contains not less than 2 mg and not more than 6 mg of magnolol, not less than 4 mg (for preparation prescribed 2 g of Perilla Herb) or not less than 6 mg (for preparation prescribed 3 g of Perilla Herb) of rosmarinic acid, and not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 1.3 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, per extract prepared with the amount specified in the Method of preparation.

**Method of preparation**

|  | 1) | 2) | 3) | 4) |
|---|---|---|---|---|
| Pinellia Tuber | 6 g | 6 g | 6 g | 6 g |
| Poria Sclerotium | 5 g | 5 g | 5 g | 5 g |
| Magnolia bark | 3 g | 3 g | 3 g | 3 g |
| Perilla Herb | 2 g | 3 g | 2 g | 2 g |
| Ginger | 1 g | 1 g | 1.3 g | 1.5 g |

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

**Description** Hangekobokuto Extract is a light brown to dark brown powder or black-brown viscous extract. It has a characteristic odor and has a bitter and astringent taste first then pungent later.

**Identification** **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of magnolol 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 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator 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: 254 nm): one of the several spots obtained from the sample solution has the same color tone and Rf value with the dark purple spot from the standard solution (Magnolia Bark).

**(2)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Separate the diethyl ether 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 rosmarinic acid 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 $\mu$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, water and formic acid (60:1:1) 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 Rf value with the dark purple spot from the standard solution (Perilla Herb).

**(3)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 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 $\mu$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 hexane and acetone (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 Rf value with the blue-green to grayish green spot from the standard solution (Ginger).

**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 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 11.0% (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 14.0%, calculated on the dried basis.

**Assay** **(1)** Magnolol—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 (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 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 magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S \times 1/8$$

$M_S$: Amount (mg) of magnolol for assay taken, calculated on the basis of the content obtained by qNMR

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wave-

length: 289 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) (50:50:1).

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

*System suitability—*

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, honokiol and magnolol 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 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

**(2)** Rosmarinic acid—Conduct this procedure using light-resistant vessels. 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 (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of rosmarinic acid for assay, dissolve in diluted methanol (7 in 10) to make exactly 200 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 rosmarinic acid in each solution.

$$\text{Amount (mg) of rosmarinic acid} = M_S \times A_T/A_S \times 1/4$$

$M_S$: Amount (mg) of rosmarinic acid for assay taken, calculated on the basis of the content obtained by qNMR

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 330 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 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of rosmarinic acid is about 11 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 rosmarinic acid 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 rosmarinic acid is not more than 1.5%.

**(3)** [6]-Gingerol—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 (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in

methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 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 [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

$M_S$: Amount (mg) of [6]-gingerol for assay taken

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 282 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 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 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 [6]-gingerol 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 [6]-gingerol is not more than 1.5%.

**Containers and storage**    Containers—Tight containers.