# Yokukansan Extract

抑肝散エキス

Yokukansan Extract contains not less than 0.15 mg of total alkaloids (rhyncophylline and hirsutine), not less than 0.6 mg and not more than 2.4 mg of saikosaponin $b_2$, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

**Method of preparation**

| | 1) | 2) |
|---|---|---|
| Japanese Angelica Root | 3 g | 3 g |
| Uncaria Hook | 3 g | 3 g |
| Cnidium Rhizome | 3 g | 3 g |
| Atractylodes Rhizome | 4 g | — |
| Atractylodes Lancea Rhizome | — | 4 g |
| Poria Sclerotium | 4 g | 4 g |
| Bupleurum Root | 2 g | 2 g |
| Glycyrrhiza | 1.5 g | 1.5 g |

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

**Description** Yokukansan Extract is a light brown to grayish brown powder or a black-brown viscous extract. It has a slightly odor, and a slightly bitter and acid taste.

**Identification** **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this layer 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 $\mu$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 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 blue-white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

**(2)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 20 mL of water and 2 mL of ammonia TS, shake, then add 20 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the solvent under low pressure (in vacuo), add 1 mL of methanol to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg each of rhyncophyllin for thin-layer chromatography and hirsutine 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 $\mu$L of the sample solution and 2 $\mu$L of the standard solution on a plate of silica gel with fluorescent indicator 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. Examine under ultraviolet light (main wavelength: 254 nm): at least one of the several spots obtained from the sample solution has the same color tone and *R*f value with one of the two dark violet spots from the standard solution (Uncaria Hook).

**(3)** For preparation prescribed Atractylodes Rhizome—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 low pressure (in vacuo), dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenoide III 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 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 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*f value with the red to purple-red spot from the standard solution (Atractylodes Rhizome).

**(4)** 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, and shake. 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 $\mu$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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

**(5)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, 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 10 $\mu$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, 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).

**(6)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 $\mu$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).

**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°C, 5 hours).

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

**Total ash** <*5.01*> Not less than 10.0%, calculated on the dried basis.

**Assay** **(1)** Total alkaloids (rhyncophylline and hirsutine)—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 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, and shake for 10 minutes, centrifuge, and remove the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, and proceed in the same manner as above. To the aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, proceed in the same manner, and repeat this procedure twice. Combine all the extracts, evaporate the solvent under low pressure (in vacuo) at not more than 40°C, dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg each of rhyncophylline for assay and hirsutine for assay, dissolve in a mixture of methanol and diluted acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add the mixture of methanol and diluted acetic acid (7:3) 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, $A_{TR}$ and $A_{TH}$, and $A_{SR}$ and $A_{SH}$, of rhyncophylline and hirsutine in each solution.

Amount (mg) of total alkaloids (rhyncophylline and hirsutine)
$$= (M_{SR} \times A_{TR}/A_{SR} + M_{SH} \times A_{TH}/A_{SH}) \times 1/50$$

$M_{SR}$: Amount (mg) of rhyncophylline for assay taken
$M_{SH}$: Amount (mg) of hirsutine for assay taken

*Operation conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 245 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: To 1 g of sodium lauryl sulfate add 600 mL of methanol, shake, then add 400 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention times of rhyncophylline and hirsutine are about 17 minutes and about 47 minutes, respectively).

*Systemic 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 peaks of rhyncophylline and hirsutine 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 rhyncophylline and hirsutine is not more than 1.5%, respectively.

**(2)** Saikosaponin $b_2$—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 diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as above, and remove the diethyl ether layer. To the aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, use saikosaponin $b_2$ 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, $A_T$ and $A_S$, of saikosaponin $b_2$ in each solution.

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

$C_S$: Concentration (mg/mL) of saikosaponin $b_2$ in saikosaponin $b_2$ standard TS for assay

*Operation 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 per minute (the retention time of saikosaponin $b_2$ is about 12 minutes).

*Systemic 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 $b_2$ 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 $b_2$ 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 diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the diethyl ether layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether 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 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 glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

$M_S$: 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 μm 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 μL 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 μL 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.