

# Suppl I, JP XVII (2017)

## Saibokuto Extract

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### Change the Origin/limits of content, Identification and Assay (3) as follows:

Saibokuto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b<sub>2</sub>, not less than 90 mg and not more than 270 mg of baicalin (C<sub>21</sub>H<sub>18</sub>O<sub>11</sub>: 446.36), and not less than 14 mg and not more than 42 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.

**Identification** (1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b<sub>2</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 10 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 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<sub>f</sub> value with the yellow fluorescent spot from the standard solution (Bupleurum Root).

(2) 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, shake, and separate the diethyl ether layer. Evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution.

Separately, dissolve 1 mg of wogonin 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 ethyl acetate, hexane and acetic acid (100) (10:10:1) 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 R<sub>f</sub> value with the yellow-brown spot from the standard solution (Scutellaria Root).

(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, shake, and separate the diethyl ether layer. Evaporate the layer under reduced pressure, 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 μL 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 R<sub>f</sub> value with the dark purple spot from the standard solution (Magnolia Bark).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid 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 10 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 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).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid 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 μL 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, 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<sub>f</sub>* value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) 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, shake, and separate the diethyl ether 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 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 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, 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 *R<sub>f</sub>* value with the dark purple spot from the standard solution (Perilla Herb).

(7) 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 layer under reduced pressure, 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 10 μL of the sample solution and 5 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 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<sub>f</sub>* value with the blue-green to grayish green spot from the standard solution (Ginger).

#### Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 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<sub>T</sub>* and *A<sub>S</sub>*, 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<sub>S</sub>*: 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. Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and baicalein 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%.

ii) 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 upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant 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. Per-

form 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.

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

$M_S$ : Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

*Operating conditions—*

Proceed as directed in the operating conditions in i).

*System suitability—*

System repeatability: Proceed as directed in the system suitability in i).

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.

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