

Shin'iseihaito Extract

辛夷清肺湯エキス

Shin'iseihaito Extract contains not less than 5 mg or not less than 20 mg of mangiferin, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 23 mg and not more than 69 mg (for preparation prescribed 1.5 g of Gardenia Fruit) or not less than 45 mg and not more than 135 mg (for preparation prescribed 3 g of Gardenia Fruit) of geniposide, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Magnolia Flower	3 g	2 g
Anemarrhena Rhizome	3 g	3 g
Lilium Bulb	3 g	3 g
Scutellaria Root	3 g	3 g
Gardenia Fruit	1.5 g	3 g
Ophiopogon Root	6 g	5 g
Gypsum	6 g	5 g
Cimicifuga Rhizome	1.5 g	1 g
Loquat Leaf	1 g	2 g

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

Description Shin'iseihaito Extract occurs as a reddish yellow to yellow-red powder. It has a slight odor, and a slightly bitter, slightly acid and slightly sweet taste.

Identification (1) To 1.0 g of Shin'iseihaito 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), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, to 1 g of powdered Magnolia Flower add 10 mL of methanol, shake, then centrifuge, and use the supernatant liquid 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 (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid 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 dark red-brown to brown spot (*R_f* value: about 0.4) from the standard solution (Magnolia Flower).

(2) To 2.0 g of Shin'iseihaito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution.

Separately, to 1 g of pulverized Anemarrhena Rhizome add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 1 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, 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 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105°C for 2 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 yellowish red to dark red spot (*R_f* value: about 0.3) from the standard solution (Anemarrhena Rhizome).

(3) To 1.0 g of Shin'iseihaito 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), then add 2 mL of diethyl ether to the residue, and use the 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 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 hexane and acetone (7:5) 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_f* value with the yellow-brown to grayish brown spot from the standard solution (Scutellaria Root).

(4) To 1.0 g of Shin'iseihaito 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 geniposide 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 μ L of the sample solution and 5 μ L of standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 1 minute: one of the several spots obtained from the sample solution has the same color tone and *R_f* value with the red-purple to dark purple spot from the standard solution (Gardenia Fruit).

(5) Place 2.0 g of Shin'iseihaito Extract in a porcelain crucible, and ignite to incinerate at 500 – 550°C. To the residue add 60 mL of water, shake, then centrifuge, and use the supernatant liquid as the sample solution. Add ammonium

95 oxalate TS to the sample solution: a white precipitate is
96 formed. The precipitate does not dissolve by the addition of
97 diluted acetic acid, but dissolves by the addition of diluted
98 hydrochloric acid (Gypsum).

99 (6) To 1.0 g of Shin'iseihaito add 10 mL of water, shake,
100 then add 10 mL of 1-butanol, shake, centrifuge, and use the
101 1-butanol layer as the sample solution. Use (*E*)-isoferulic
102 acid-(*E*)-ferulic acid TS for thin-layer chromatography as the
103 standard solution. Perform the test with these solutions as di-
104 rected under Thin-layer Chromatography <2.03>. Spot 10 μ L
105 of the sample solution and 2 μ L of the standard solution on a
106 plate of silica gel for thin-layer chromatography. Develop the
107 plate with a mixture of ethyl acetate, acetone and water
108 (20:12:3) to a distance of about 7 cm, and air-dry the plate.
109 Spray evenly sulfuric acid on the plate, and heat the plate at
110 105°C for 5 minutes, and examine under ultraviolet light
111 (main wavelength: 365 nm): one of the several spots obtained
112 from the sample solution has the same color tone and *R_f* value
113 with the light yellow-white to yellow-green fluorescent spot
114 from the standard solution (Cimicifuga Rhizome).

115 **Purity** (1) Heavy metals <1.07>—Prepare the test solu-
116 tion with 1.0 g of Shin'iseihaito as directed under the Extracts
117 (4), and perform the test (not more than 30 ppm).

118 (2) Arsenic <1.11>—Prepare the test solution with 0.67
119 g of Shin'iseihaito according to Method 3, and perform the
120 test (not more than 3 ppm).

121 **Loss on drying** <2.41> Not more than 9.0% (1 g, 105°C, 5
122 hours).

123 **Total ash** <5.01> Not more than 14.0%.

124 **Assay** (1) Mangiferin—Weigh accurately about 0.5 g of
125 Shin'iseihaito, add exactly 50 mL of diluted methanol (1 in
126 2), shake for 15 minutes, then centrifuge, and use the super-
127 natant liquid as the sample solution. Separately, weigh accu-
128 rately about 10 mg of mangiferin for assay, dissolve in di-
129 luted methanol (1 in 2) to make exactly 200 mL, and use this
130 solution as the standard solution. Perform the test with ex-
131 actly 10 μ L each of the sample solution and standard solution
132 as directed under Liquid Chromatography <2.01> according to
133 the following conditions, and determine the peak areas, *A_T*
134 and *A_S*, of mangiferin in each solution.

135 Amount (mg) of mangiferin = $M_S \times A_T / A_S \times 1/4$

136 *M_S*: Amount (mg) of mangiferin for assay taken, calculated
137 on the basis of the content obtained by qNMR

138 **Operating conditions**—

139 Detector: An ultraviolet absorption photometer (wave-
140 length: 367 nm).

141 Column: A stainless steel column 4.6 mm in inside diam-
142 eter and 15 cm in length, packed with octadecylsilanized sil-
143 ica gel for liquid chromatography (5 μ m in particle diameter).

144 Column temperature: A constant temperature of about
145 40°C.

146 Mobile phase: A mixture of water, acetonitrile and phos-
147 phoric acid (1780:220:1).

148 Flow rate: 1.0 mL per minute.

149 **System suitability**—

150 System performance: When the procedure is run with 10
151 μ L of the standard solution under the above operating condi-
152 tions, the number of theoretical plates and the symmetry fac-
153 tor of the peak of mangiferin are not less than 5000 and not
154 more than 1.5, respectively.

155 System repeatability: When the test is repeated 6 times
156 with 10 μ L of the standard solution under the above operating
157 conditions, the relative standard deviation of the peak area of
158 mangiferin is not more than 1.5%.

159 (2) Baicalin—Weigh accurately about 0.1 g of Shin'isei-
160 haito, add exactly 50 mL of diluted methanol (7 in 10), shake
161 for 15 minutes, then filter, and use the filtrate as the sample
162 solution. Separately, weigh accurately about 10 mg of Bai-
163 calin RS (separately determine the water <2.48> by coulo-
164 metric titration, using 10 mg), dissolve in methanol to make
165 exactly 100 mL. Pipet 5 mL of this solution, add diluted
166 methanol (7 in 10) to make exactly 10 mL, and use this solu-
167 tion as the standard solution. Perform the test with exactly 10
168 μ L each of the sample solution and standard solution as di-
169 rected under Liquid Chromatography <2.01> according to the
170 following conditions, and determine the peak areas, *A_T* and
171 *A_S*, of baicalin in each solution.

172 Amount (mg) of baicalin (C₂₁H₁₈O₁₁)
173 = $M_S \times A_T / A_S \times 1/4$

174 *M_S*: Amount (mg) of Baicalin RS taken, calculated on the
175 anhydrous basis

176 **Operating conditions**—

177 Detector: An ultraviolet absorption photometer (wave-
178 length: 277 nm).

179 Column: A stainless steel column 4.6 mm in inside diam-
180 eter and 15 cm in length, packed with octadecylsilanized sil-
181 ica gel for liquid chromatography (5 μ m in particle diameter).

182 Column temperature: A constant temperature of about
183 40°C.

184 Mobile phase: A mixture of diluted phosphoric acid (1 in
185 200) and acetonitrile (19:6).

186 Flow rate: 1.0 mL per minute.

187 **System suitability**—

188 System performance: When the procedure is run with 10
189 μ L of the standard solution under the above operating condi-
190 tions, the number of theoretical plates and the symmetry fac-
191 tor of the peak of baicalin are not less than 5000 and not more
192 than 1.5, respectively.

193 System repeatability: When the test is repeated 6 times
194 with 10 μ L of the standard solution under the above operating

195 conditions, the relative standard deviation of the peak area of
196 baicalin is not more than 1.5%.

197 **(3) Geniposide** — Weigh accurately about 0.5 g of
198 Shin'iseihaito, add exactly 50 mL of diluted methanol (1 in
199 2), shake for 15 minutes, then centrifuge, and use the super-
200 natant liquid as the sample solution. Separately, weigh accu-
201 rately about 10 mg of geniposide for assay, dissolve in diluted
202 methanol (1 in 2) to make exactly 100 mL, and use this solu-
203 tion as the standard solution. Perform the test with exactly 10
204 μL each of the sample solution and standard solution as di-
205 rected under Liquid Chromatography <2.01> according to the
206 following conditions, and determine the peak areas, A_T and
207 A_S , of geniposide in each solution.

208 Amount (mg) of geniposide = $M_S \times A_T / A_S \times 1/2$

209 M_S : Amount (mg) of geniposide for assay taken, calculated
210 on the basis of the content obtained by qNMR

211 *Operating conditions*—

212 Detector: An ultraviolet absorption photometer (wave-
213 length: 240 nm).

214 Column: A stainless steel column 4.6 mm in inside diam-
215 eter and 15 cm in length, packed with octadecylsilanized sil-
216 ica gel for liquid chromatography (5 μm in particle diameter).

217 Column temperature: A constant temperature of about
218 40°C.

219 Mobile phase: A mixture of water, acetonitrile and phos-
220 phoric acid (900:100:1).

221 Flow rate: 1.0 mL per minute.

222 *System suitability*—

223 System performance: When the procedure is run with 10
224 μL of the standard solution under the above operating condi-
225 tions, the number of theoretical plates and the symmetry fac-
226 tor of the peak of geniposide are not less than 5000 and not
227 more than 1.5, respectively.

228 System repeatability: When the test is repeated 6 times
229 with 10 μL of the standard solution under the above operating
230 conditions, the relative standard deviation of the peak area of
231 geniposide is not more than 1.5%.

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

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