

1 Saikokeishikankyoto Extract

2 柴胡桂枝乾姜湯エキス

3 Saikokeishikankyoto Extract contains not less than
4 1.4 mg and not more than 5.6 mg of saikosaponin b₂,
5 not less than 78 mg and not more than 234 mg of bai-
6 calin (C₂₁H₁₈O₁₁: 446.36), and not less than 15 mg and
7 not more than 45 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆:
8 822.93), per extract prepared with the amount speci-
9 fied in the Method of preparation.

10 Method of preparation

	1)	2)
Bupleurum Root	6 g	6 g
Cinnamon Bark	3 g	3 g
Scutellaria Root	3 g	3 g
Oyster Shell	3 g	3 g
Processed Ginger	2 g	3 g
Glycyrrhiza	2 g	2 g
Trichosanthes Root	3 g	4 g

11
12 Prepare a dry extract or viscous extract as directed under
13 Extracts, according to the prescription 1) or 2), using the
14 crude drugs shown above.

15 **Description** The dry extract occurs as a light yel-
16 low-brown to brown powder. It has a characteristic odor and
17 a hot, bitter and slightly sweet taste.

18 The viscous extract occurs as a black-brown viscous liq-
19 uid. It has a characteristic odor and a bitter, hot and slightly
20 sweet taste, followed by an astringent aftertaste.

21 **Identification (1)** To 1.0 g of the dry extract (or 3.0 g of
22 the viscous extract) add 10 mL of water, shake, add 10 mL
23 of 1-butanol, shake, centrifuge, and use the 1-butanol layer
24 as the sample solution. Separately, dissolve 1 mg of sai-
25 kosaponin b₂ for thin-layer chromatography in 1 mL of
26 methanol, and use this solution as the standard solution.
27 Perform the test with these solutions as directed under
28 Thin-layer Chromatography <2.03>. Spot 5 μL of the sample
29 solution and 2 μL of the standard solution on a plate of sili-
30 ca gel for thin-layer chromatography. Develop the plate
31 with a mixture of ethyl acetate, ethanol (99.5) and water
32 (8:2:1) to a distance of about 7 cm, and air-dry the plate.
33 Spray evenly 4-dimethylaminobenzaldehyde TS for spray-
34 ing on the plate, and heat the plate at 105°C for 5 minutes.
35 Examine under ultraviolet light (main wavelength: 365 nm):
36 one of the several spots obtained from the sample solution
37 has the same color tone and R_f value with the yellow fluo-
38 rescent spot from the standard solution (Bupleurum Root).

39 **(2)** Perform the test according to the following i) or ii)
40 (Cinnamon Bark).

41 i) Put 10 g of dry extract (or 30 g of the viscous extract)
42 in a 300-mL of hard-glass flask, add 100 mL of water and 1
43 mL of silicone resin, connect the apparatus for essential oil

44 determination, and heat to boil under a reflux condenser.
45 The graduated tube of the apparatus is to be previously
46 filled with water to the standard line, and 2 mL of hexane is
47 added to the graduated tube. After heating under reflux for 1
48 hour, separate the hexane layer, and use this solution as the
49 sample solution. Separately, dissolve 1 mg of
50 (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL
51 of methanol, and use this solution as the standard solution.
52 Perform the test with these solutions as directed under
53 Thin-layer Chromatography <2.03>. Spot 20 μL of the sam-
54 ple solution and 2 μL of the standard solution on a plate of
55 silica gel for thin-layer chromatography. Develop the plate
56 with a mixture of hexane, diethyl ether and methanol
57 (15:5:1) to a distance of about 7 cm, and air-dry the plate.
58 Spray evenly 2,4-dinitrophenylhydrazine TS on the plate:
59 one of the several spots obtained from the sample solution
60 has the same color tone and R_f value with the yellow-orange
61 to orange spot from the standard solution.

62 ii) To 2.0 g of dry extract (or 6.0 g of the viscous ex-
63 tract) add 10 mL of water, shake, add 5 mL of hexane,
64 shake, centrifuge, and use the hexane layer as the sample
65 solution. Separately, dissolve 1 mg of
66 (*E*)-2-methoxycinnamaldehyde for thin-layer chromatog-
67 raphy in 1 mL of methanol, and use this solution as the
68 standard solution. Perform the test with these solutions as
69 directed under Thin-layer Chromatography <2.03>. Spot 20
70 μL of the sample solution and 2 μL of the standard solution
71 on a plate of silica gel for thin-layer chromatography. De-
72 velop the plate with a mixture of hexane and ethyl acetate
73 (2:1) to a distance of about 7 cm, and air-dry the plate. Ex-
74 amine under ultraviolet light (main wavelength: 365 nm):
75 one of the several spots obtained from the sample solution
76 has the same color tone and R_f value with the blue-white
77 fluorescent spot from the standard solution.

78 **(3)** To 1.0 g of the dry extract (or 3.0 g of the viscous
79 extract) add 10 mL of water, shake, add 25 mL of diethyl
80 ether, and shake. Separate the diethyl ether layer, evaporate
81 the solvent under a low pressure (vacuum), add 2 mL of
82 diethyl ether to the residue, and use this solution as the
83 sample solution. Separately, dissolve 1 mg of wogonin for
84 thin-layer chromatography in 1 mL of methanol, and use
85 this solution as the standard solution. Perform the test with
86 these solutions as directed under Thin-layer Chromatog-
87 raphy <2.03>. Spot 10 μL of the sample solution and 2 μL of
88 the standard solution on a plate of silica gel for thin-layer
89 chromatography. Develop the plate with a mixture of hex-
90 ane and acetone (7:5) to a distance of about 7 cm, and
91 air-dry the plate. Spray evenly iron (III) chloride-methanol
92 TS on the plate: one of the several spots obtained from the
93 sample solution has the same color tone and R_f value with
94 the yellow-brown to grayish brown spot from the standard
95 solution (Scutellaria Root).

96 (4) To 1.0 g of the dry extract (or 3.0 g of the viscous
97 extract) add 10 mL of water, shake, add 25 mL of diethyl
98 ether, and shake. Separate the diethyl ether layer, evaporate
99 the solvent under a low pressure (vacuum), add 2 mL of
100 diethyl ether to the residue, and use this solution as the
101 sample solution. Separately, dissolve 1 mg of [6]-shogaol
102 for thin-layer chromatography in 1 mL of methanol, and use
103 this solution as the standard solution. Perform the test with
104 these solutions as directed under Thin-layer Chromatog-
105 raphy <2.03>. Spot 20 μL of the sample solution and 5 μL
106 of the standard solution on a plate of silica gel for thin-layer
107 chromatography. Develop the plate with a mixture of ethyl
108 acetate and hexane (1:1) to a distance of about 7 cm, and
109 air-dry the plate. Spray evenly 4-dimethylaminobenzalde-
110 hyde TS for spraying on the plate, heat the plate at 105°C
111 for 5 minutes, allow to cool, and spray water: one of the
112 several spots obtained from the sample solution has the
113 same color tone and R_f value with the blue-green to grayish
114 green spot from the standard solution (Processed Ginger).

115 (5) To 1.0 g of the dry extract (or 3.0 g of the viscous
116 extract) add 10 mL of water, shake, add 10 mL of 1-butanol,
117 shake, centrifuge, and use the 1-butanol layer as the sample
118 solution. Separately, dissolve 1 mg of liquiritin for
119 thin-layer chromatography in 1 mL of methanol, and use
120 this solution as the standard solution. Perform the test with
121 these solutions as directed under Thin-layer Chromatog-
122 raphy <2.03>. Spot 1 μL each of the sample solution and
123 standard solution on a plate of silica gel for thin-layer
124 chromatography. Develop the plate with a mixture of ethyl
125 acetate, methanol and water (20:3:2) to a distance of about 7
126 cm, and air-dry the plate. Spray evenly dilute sulfuric acid
127 on the plate, heat the plate at 105°C for 5 minutes, and ex-
128 amine under ultraviolet light (main wavelength: 365 nm):
129 one of the several spots obtained from the sample solution
130 has the same color tone and R_f value with the yellow to
131 yellow-green fluorescent spot from the standard solution
132 (Glycyrrhiza).

133 **Purity (1)** Heavy metals <1.07>—Prepare the test solu-
134 tion with 1.0 g of the dry extract (or an amount of the vis-
135 cious extract, equivalent to 1.0 g of the dried substance) as
136 directed under the Extracts (4), and perform the test (not
137 more than 30 ppm).

138 (2) Arsenic <1.11>—Prepare the test solution with 0.67
139 g of the dry extract (or an amount of the viscous extract,
140 equivalent to 0.67 g of the dried substance) according to
141 Method 3, and perform the test (not more than 3 ppm).

142 **Loss on drying <2.41>** The dry extract: Not more than
143 9.5% (1 g, 105°C, 5 hours).

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

146 **Total ash <5.01>** Not more than 13.0%, calculated on the
147 dried basis.

148 **Assay (1)** Saikosaponin b_2 —Weigh accurately about 0.5
149 g of the dry extract (or an amount of the viscous extract,
150 equivalent to about 0.5 g of the dried substance), add 20 mL
151 of diethyl ether and 10 mL of water, and shake for 10
152 minutes. Centrifuge, remove the diethyl ether layer, add 20
153 mL of diethyl ether, proceed in the same manner as de-
154 scribed above, and remove the diethyl ether layer. To the
155 water layer add 10 mL of methanol, shake for 30 minutes,
156 centrifuge, and separate the supernatant liquid. To the resi-
157 due add 20 mL of diluted methanol (1 in 2), shake for 5
158 minutes, centrifuge, and separate the supernatant liquid.
159 Combine these supernatant liquids, add diluted methanol (1
160 in 2) to make exactly 50 mL, and use this solution as the
161 sample solution. Separately, use saikosaponin b_2 standard
162 TS for assay as the standard solution. Perform the test with
163 exactly 10 μL each of the sample solution and standard so-
164 lution as directed under Liquid Chromatography <2.01>
165 according to the following conditions, and determine the
166 peak areas, A_T and A_S , of saikosaponin b_2 in each solution.

$$167 \text{ Amount (mg) of saikosaponin } b_2 = C_S \times A_T / A_S \times 50$$

168 C_S : Concentration (mg/mL) of saikosaponin b_2 in sai-
169 kosaponin b_2 standard TS for assay

170 *Operating conditions—*

171 Detector: An ultraviolet absorption photometer (wave-
172 length: 254 nm).

173 Column: A stainless steel column 4.6 mm in inside diam-
174 eter and 15 cm in length, packed with octadecylsilanized
175 silica gel for liquid chromatography (5 μm in particle diam-
176 eter).

177 Column temperature: A constant temperature of about
178 40°C.

179 Mobile phase: A mixture of 0.05 mol/L sodium dihydro-
180 gen phosphate TS and acetonitrile (5:3).

181 Flow rate: 1.0 mL per minute.

182 *System suitability—*

183 System performance: When the procedure is run with 10
184 μL of the standard solution under the above operating con-
185 ditions, the number of theoretical plates and the symmetry
186 factor of the peak of saikosaponin b_2 are not less than 5000
187 and not more than 1.5, respectively.

188 System repeatability: When the test is repeated 6 times
189 with 10 μL of the standard solution under the above operat-
190 ing conditions, the relative standard deviation of the peak
191 area of saikosaponin b_2 is not more than 1.5%.

192 (2) Baicalin—Weigh accurately about 0.1 g of the dry
193 extract (or an amount of the viscous extract, equivalent to
194 about 0.1 g of the dried substance), add exactly 50 mL of
195 diluted methanol (7 in 10), shake for 15 minutes, filter, and

196 use the filtrate as the sample solution. Separately, weigh
197 accurately about 10 mg of Baicalin RS (separately deter-
198 mine the water <2.48> by coulometric titration, using 10 mg),
199 and dissolve in methanol to make exactly 100 mL. Pipet 5
200 mL of this solution, add diluted methanol (7 in 10) to make
201 exactly 10 mL, and use this solution as the standard solution.
202 Perform the test with exactly 10 μ L each of the sample so-
203 lution and standard solution as directed under Liquid Chro-
204 matography <2.01> according to the following conditions,
205 and determine the peak areas, A_T and A_S , of baicalin in each
206 solution.

207 Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$)

$$208 = M_S \times A_T / A_S \times 1/4$$

209 M_S : Amount (mg) of Baicalin RS taken, calculated on the
210 anhydrous basis

211 *Operating conditions—*

212 Detector: An ultraviolet absorption photometer (wave-
213 length: 277 nm).

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

218 Column temperature: A constant temperature of about
219 40°C.

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

222 Flow rate: 1.0 mL per minute.

223 *System suitability—*

224 System performance: When the procedure is run with 10
225 μ L of the standard solution under the above operating con-
226 ditions, the number of theoretical plates and the symmetry
227 factor of the peak of baicalin are not less than 5000 and not
228 more than 1.5, respectively.

229 System repeatability: When the test is repeated 6 times
230 with 10 μ L of the standard solution under the above operat-
231 ing conditions, the relative standard deviation of the peak
232 area of baicalin is not more than 1.5%.

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

235 i) Weigh accurately about 0.5 g of the dry extract (or an
236 amount of the viscous extract, equivalent to about 0.5 g of
237 the dried substance), add exactly 50 mL of diluted methanol
238 (1 in 2), shake for 15 minutes, filter, and use the filtrate as
239 the sample solution. Separately, weigh accurately about 10
240 mg of Glycyrrhizic Acid RS (separately determine the water
241 <2.48> by coulometric titration, using 10 mg), dissolve in
242 diluted methanol (1 in 2) to make exactly 100 mL, and use
243 this solution as the standard solution. Perform the test with
244 exactly 10 μ L each of the sample solution and standard so-
245 lution as directed under Liquid Chromatography <2.01>

246 according to the following conditions, and determine the
247 peak areas, A_T and A_S , of glycyrrhizic acid in each solution.

248 Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)

$$249 = M_S \times A_T / A_S \times 1/2$$

250 M_S : Amount (mg) of Glycyrrhizic Acid RS taken, calcu-
251 lated on the anhydrous basis

252 *Operating conditions—*

253 Detector: An ultraviolet absorption photometer (wave-
254 length: 254 nm).

255 Column: A stainless steel column 4.6 mm in inside diam-
256 eter and 15 cm in length, packed with octadecylsilanized
257 silica gel for liquid chromatography (5 μ m in particle diam-
258 eter).

259 Column temperature: A constant temperature of about
260 40°C.

261 Mobile phase: Dissolve 3.85 g of ammonium acetate in
262 720 mL of water, and add 5 mL of acetic acid (100) and 280
263 mL of acetonitrile.

264 Flow rate: 1.0 mL per minute.

265 *System suitability—*

266 System performance: Dissolve 5 mg of monoammonium
267 glycyrrhizinate for resolution check in 20 mL of dilute eth-
268 anol. When the procedure is run with 10 μ L of this solution
269 under the above operating conditions, the resolution be-
270 tween the peak having the relative retention time of about
271 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is
272 not less than 1.5%. Dissolve 1 mg of (*E*)-cinnamaldehyde
273 for thin-layer chromatography and 1 mg of baicalein for
274 resolution check in 50 mL of methanol. To 2 mL of this
275 solution add 2 mL of the standard solution. When the pro-
276 cedure is run with 10 μ L of this solution under the above
277 operating conditions, two peaks other than glycyrrhizic acid
278 are observed with the resolutions between the peak of
279 glycyrrhizic acid and each of the two peaks being not less
280 than 1.5.

281 System repeatability: When the test is repeated 6 times
282 with 10 μ L of the standard solution under the above operat-
283 ing conditions, the relative standard deviation of the peak
284 area of glycyrrhizic acid is not more than 1.5%.

285 ii) Weigh accurately about 0.5 g of the dry extract (or
286 an amount of the viscous extract, equivalent to about 0.5 g
287 of the dried substance), add 20 mL of diethyl ether and 10
288 mL of water, and shake for 10 minutes. Centrifuge, remove
289 the diethyl ether layer, add 20 mL of diethyl ether, proceed
290 in the same manner as described above, and remove the
291 diethyl ether layer. To the water layer add 10 mL of metha-
292 nol, shake for 30 minutes, centrifuge, and separate the su-
293 pernatant liquid. To the residue add 20 mL of diluted meth-
294 anol (1 in 2), shake for 5 minutes, centrifuge, and separate
295 the supernatant liquid. Combine these supernatant liquids,
296 add diluted methanol (1 in 2) to make exactly 50 mL, and

297 use this solution as the sample solution. Separately, weigh
298 accurately about 10 mg of Glycyrrhizic Acid RS (separately
299 determine the water <2.48> by coulometric titration, using
300 10 mg), dissolve in diluted methanol (1 in 2) to make ex-
301 actly 100 mL, and use this solution as the standard solution.
302 Perform the test with exactly 10 μL each of the sample so-
303 lution and standard solution as directed under Liquid Chro-
304 matography <2.01> according to the following conditions,
305 and determine the peak areas, A_T and A_S , of glycyrrhizic
306 acid in each solution.

$$\begin{aligned} 307 \quad & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}\text{)} \\ 308 \quad & = M_S \times A_T / A_S \times 1/2 \end{aligned}$$

309 M_S : Amount (mg) of Glycyrrhizic Acid RS taken, calcu-
310 lated on the anhydrous basis

311 *Operating conditions*—

312 Proceed as directed in the operating conditions in i).

313 *System suitability*—

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

316 System performance: Dissolve 5 mg of monoammonium
317 glycyrrhizinate for resolution check in 20 mL of dilute eth-
318 anol. When the procedure is run with 10 μL of this solution
319 under the above operating conditions, the resolution be-
320 tween the peak having the relative retention time of about
321 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is
322 not less than 1.5.

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