

1 Byakkokaninjinto Extract

2 白虎加人参湯エキス

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4 Byakkokaninjinto Extract contains not less than 9
5 mg and not more than 36 mg of mangiferin, not less
6 than 13 mg and not more than 39 mg of glycyrrhizic
7 acid (C₄₂H₆₂O₁₆: 822.93), and not less than 0.9 mg
8 (for preparation prescribed 1.5 g of Ginseng) or not
9 less than 1.8 mg (for preparation prescribed 3 g of
10 Ginseng) of ginsenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29),
11 per extract prepared with the amount specified in the
12 Method of preparation.

13 Method of preparation

14

	1)	2)
Anemarrhena Rhizome	5 g	5 g
Gypsum	15 g	15 g
Glycyrrhiza	2 g	2 g
Brown Rice	8 g	8 g
Ginseng	1.5 g	3 g

15

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

19 **Description** Byakkokaninjinto Extract occurs as a very
20 pale yellow-brown to light brown powder, or blackish
21 brown viscous extract. It has a slight odor, and has a
22 slightly sweet and slightly bitter taste.

23 **Identification** (1) To 2.0 g of the dry extract (or 6.0 g
24 of the viscous extract) add 10 mL of sodium hydroxide TS,
25 shake, then add 5 mL of 1-butanol, shake, centrifuge, and
26 use the supernatant liquid as the sample solution. Sepa-
27 rately, to 1 g of pulverized Anemarrhena Rhizome add 10
28 mL of water, shake, then add 10 mL of 1-butanol, shake,
29 centrifuge, and use the supernatant liquid as the standard
30 solution. Perform the test with these solutions as directed
31 under Thin-layer Chromatography <2.03>. Spot 5 μL of the
32 sample solution and 1 μL of the standard solution on a plate
33 of silica gel for thin-layer chromatography. Develop the
34 plate with a mixture of ethyl acetate, 1-propanol, water and
35 acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and
36 air-dry the plate. Spray evenly 4-dimethylaminobenzalde-
37 hyde TS for spraying on the plate, and heat at 105°C for 2
38 minutes, and allow to cool: one of the several spots ob-
39 tained from the sample solution has the same color tone and
40 R_f value with the yellowish red to dark red spot (at an R_f
41 value of about 0.3) from the standard solution (Anemar-
42 rhena Rhizome).

43 (2) Place 2.0 g of the dry extract (or 6.0 g of the viscous
44 extract) in a porcelain crucible, and ignite to incinerate at
45 500 – 550°C. Add 60 mL of water to the residue, shake,
46 centrifuge, and use the supernatant as the sample solution.

47 Add ammonium oxalate TS to the sample solution: a white
48 precipitate is formed. The precipitate does not dissolve in
49 diluted acetic acid, but dissolves on the addition of diluted
50 hydrochloric acid (Gypsum).

51 (3) To 1.0 g of the dry extract (or 3.0 g of the viscous
52 extract) add 10 mL of water, shake, then add 10 mL of 1-
53 butanol, shake, centrifuge, and use the supernatant liquid as
54 the sample solution. Separately, dissolve 1 mg of liquiritin
55 for thin-layer chromatography in 1 mL of methanol, and
56 use this solution as the standard solution. Perform the test
57 with these solutions as directed under Thin-layer Chroma-
58 tography <2.03>. Spot 1 μL each of the sample solution and
59 standard solution on a plate of silica gel for thin-layer chro-
60 matography. Develop the plate with a mixture of ethyl ac-
61 etate, methanol and water (20:3:2) to a distance of about 7
62 cm, and air-dry the plate. Spray evenly dilute sulfuric acid
63 on the plate, heat at 105°C for 5 minutes, and examine un-
64 der ultraviolet light (main wavelength: 365 nm): one of the
65 several spots obtained from the sample solution has the
66 same color tone and R_f value with the yellow-green fluo-
67 rescent spot from the standard solution (Glycyrrhiza).

68 (4) To 5.0 g of dry extract (or 15 g of the viscous ex-
69 tract) add 15 mL of water, shake, then add 5 mL of diethyl
70 ether, shake, centrifuge, and use the supernatant liquid as
71 the sample solution. Separately, dissolve 1 mg of cyclo-
72 artenyl ferulate for thin-layer chromatography in 1 mL
73 of ethyl acetate, and use this solution as the standard solu-
74 tion. Perform the test with these solutions as directed under
75 Thin-layer Chromatography <2.03>. Spot 30 μL of the sam-
76 ple solution and 5 μL of the standard solution on a plate of
77 silica gel for thin-layer chromatography. Develop the plate
78 with a mixture of hexane, acetone and acetic acid (100)
79 (50:20:1) to a distance of about 7 cm, and air-dry the plate.
80 Spray evenly a mixture of sulfuric acid and ethanol (99.5)
81 (1:1), heat at 105°C for 5 minutes, and examine under ul-
82 traviolet light (main wavelength: 365 nm): one of the sev-
83 eral spots obtained from the sample solution has the same
84 color tone and R_f value with the light yellow-white to yel-
85 low fluorescent spot from the standard solution. (Brown
86 Rice).

87 (5) To 2.0 g of dry extract (or 6.0 g of the viscous ex-
88 tract) add 10 mL of sodium hydroxide TS, shake, then add
89 5 mL of 1-butanol, shake, centrifuge, and use the superna-
90 tant liquid as the sample solution. Separately, dissolve 1 mg
91 of ginsenoside Rb₁ for thin-layer chromatography in 1 mL
92 of methanol, and use this solution as the standard solution.
93 Perform the test with these solutions as directed under
94 Thin-layer Chromatography <2.03>. Spot 10 μL of the sam-
95 ple solution and 5 μL of the standard solution on a plate of
96 silica gel for thin-layer chromatography. Develop the plate
97 with a mixture of ethyl acetate, 1-propanol, water and ace-
98 tic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-

99 dry the plate. Spray evenly vanillin-sulfuric acid-ethanol
100 TS for spraying on the plate, heat at 105°C for 5 minutes,
101 and allow to cool: one of the several spots obtained from
102 the sample solution has the same color tone and *R_f* value
103 with the blue-purple to dark purple spot from the standard
104 solution (Ginseng).

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

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

114 **Loss on drying** <2.41> The dry extract: Not more than
115 10.0% (1 g, 105°C, 5 hours).

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

118 **Total ash** <5.01> Not more than 20.0%, calculated on the
119 dried basis.

120 **Assay (1)** Mangiferin Weigh accurately about 0.5 g
121 of the dry extract (or an amount of the viscous extract,
122 equivalent to about 0.5 g of the dried substance), add ex-
123 actly 50 mL of diluted methanol (1 in 2), shake for 15
124 minutes, centrifuge, and use the supernatant as the sample
125 solution. Separately, weigh accurately about 10 mg of man-
126 giferin for assay, dissolve in diluted methanol (1 in 2) to
127 make exactly 200 mL, and use this solution as the standard
128 solution. Perform the test with exactly 10 µL each of the
129 sample solution and standard solution as directed under
130 Liquid Chromatography <2.01> according to the following
131 conditions, and determine the peak areas, *A_T* and *A_S*, of
132 mangiferin in each solution.

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

134 *M_S*: Amount (mg) of mangiferin for assay taken, calcu-
135 lated on the basis of the content obtained by qNMR

136 **Operating conditions**—

137 **Detector:** An ultraviolet absorption photometer
138 (wavelength: 367 nm).

139 **Column:** A stainless steel column 4.6 mm in inside
140 diameter and 15 cm in length, packed with
141 octadecylsilanized silica gel for liquid chromatography (5
142 µm in particle diameter).

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

145 **Mobile phase:** A mixture of water, acetonitrile and
146 phosphoric acid (1780:220:1).

147 **Flow rate:** 1.0 mL per minute.

148 **System suitability**—

149 **System performance:** When the procedure is run with 10
150 µL of the standard solution under the above operating
151 conditions, the number of theoretical plates and the
152 symmetry factor of the peak of mangiferin are not less than
153 5000 and not more than 1.5, respectively.

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

158 **(2)** Glycyrrhizic acid—Weigh accurately about 0.5 g
159 of the dry extract (or an amount of the viscous extract,
160 equivalent to about 0.5 g of dried substance), add exactly
161 50 mL of diluted methanol (1 in 2), shake for 15 minutes,
162 filter, and use the filtrate as the sample solution. Separately,
163 weigh accurately about 10 mg of Glycyrrhizic Acid RS
164 (separately determine the water <2.48> by coulometric ti-
165 tration, using 10 mg), dissolve in diluted methanol (1 in 2)
166 to make exactly 100 mL, and use this solution as the stand-
167 ard solution. Perform the test with exactly 10 µL each of
168 the sample solution and standard solution as directed under
169 Liquid Chromatography <2.01> according to the following
170 conditions, and determine the peak areas, *A_T* and *A_S*, of
171 glycyrrhizic acid in each solution.

172 Amount (mg) of glycyrrhizic acid (C₄₂H₆₂O₁₆)
173 = $M_S \times A_T / A_S \times 1/2$

174 *M_S*: Amount (mg) of Glycyrrhizic Acid RS taken, calcu-
175 lated on the anhydrous basis

176 **Operating conditions**—

177 **Detector:** An ultraviolet absorption photometer
178 (wavelength: 254 nm).

179 **Column:** A stainless steel column 4.6 mm in inside
180 diameter and 15 cm in length, packed with
181 octadecylsilanized silica gel for liquid chromatography (5
182 µm in particle diameter).

183 **Column temperature:** A constant temperature of about
184 40°C.

185 **Mobile phase:** Dissolve 3.85 g of ammonium acetate in
186 720 mL of water, and add 5 mL of acetic acid (100) and
187 280 mL of acetonitrile.

188 **Flow rate:** 1.0 mL per minute.

189 **System suitability**—

190 **System performance:** Dissolve 5 mg of monoammonium
191 glycyrrhizinate for resolution check in 20 mL of dilute
192 ethanol. When the procedure is run with 10 µL of this
193 solution under the above operating conditions, the
194 resolution between the peak having the relative retention
195 time of about 0.9 to glycyrrhizic acid and the peak of
196 glycyrrhizic acid is not less than 1.5.

197 **System repeatability:** When the test is repeated 6 times
198 with 10 µL of the standard solution under the above

199 operating conditions, the relative standard deviation of the
200 peak area of glycyrrhizic acid is not more than 1.5%.

201 **(3) Ginsenoside Rb₁**—Weigh accurately about 1 g of
202 the dry extract (or an amount of the viscous extract, equiv-
203 alent to about 1 g of dried substance), add 25 mL of diluted
204 methanol (3 in 5), shake for 30 minutes, then allow to stand,
205 and separate the supernatant liquid. To the residue add 8
206 mL of water, shake for 15 minutes, then add 12 mL of
207 methanol, shake for 15 minutes, centrifuge, and separate
208 the supernatant liquid. Combine all the supernatant liquids,
209 and add diluted methanol (3 in 5) to make exactly 50 mL.
210 Pipet 10 mL of this solution, add 3 mL of sodium hydroxide
211 TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L
212 hydrochloric acid TS, and add water to make exactly 20 mL.
213 Apply exactly 10 mL of this solution to a column [about 10
214 mm in inside diameter, packed with 0.36 g of octadecylsi-
215 lanized silica gel for pre-treatment (55 – 105 μm in particle
216 size), and washed just before using with methanol and then
217 diluted methanol (3 in 10)], and wash the column in se-
218 quence with 2 mL of diluted methanol (3 in 10), 1 mL of
219 sodium carbonate TS and 10 mL of diluted methanol (3 in
220 10). Finally, elute with methanol to collect exactly 5 mL,
221 and use this solution as the sample solution. Separately,
222 weigh accurately about 10 mg of Ginsenoside Rb₁ RS (sep-
223 arately determine the water <2.48> by coulometric titration,
224 using 10 mg), and dissolve in methanol to make exactly 100
225 mL. Pipet 10 mL of this solution, add methanol to make
226 exactly 50 mL, and use this solution as the standard solu-
227 tion. Perform the test with exactly 20 μL each of the sample
228 solution and standard solution as directed under Liquid
229 Chromatography <2.01> according to the following condi-
230 tions, and determine the peak areas, A_T and A_S, of ginseno-
231 side Rb₁ in each solution.

$$232 \quad \text{Amount (mg) of ginsenoside Rb}_1 \text{ (C}_{54}\text{H}_{92}\text{O}_{23}\text{)} \\ 233 \quad = M_S \times A_T / A_S \times 1 / 10$$

234 M_S: Amount (mg) of Ginsenoside Rb₁ RS taken, calcu-
235 lated on the anhydrous basis

236 For the preparation 1)

237 *Operating conditions*—

238 Detector: An ultraviolet absorption photometer (wave-
239 length: 203 nm).

240 Column: A stainless steel column 4.6 mm in inside di-
241 ameter and 15 cm in length, packed with carbamoyl group
242 bound silica gel for liquid chromatography (3.5 μm in par-
243 ticle diameter).

244 Column temperature: A constant temperature of about
245 60°C.

246 Mobile phase: A mixture of acetonitrile, water and phos-
247 phoric acid (1700:300:1).

248 Flow rate: 1.0 mL per minute.

249 *System suitability*—

250 System performance: When the procedure is run with 20
251 μL of the standard solution under the above operating con-
252 ditions, the number of theoretical plates and the symmetry
253 factor of the peak of ginsenoside Rb₁ are not less than 5000
254 and not more than 1.5, respectively.

255 System repeatability: When the test is repeated 6 times
256 with 20 μL of the standard solution under the above oper-
257 ating conditions, the relative standard deviation of the peak
258 area of ginsenoside Rb₁ is not more than 1.5%.

259 For the preparation 2)

260 *Operating conditions*—

261 Detector, column temperature, and flow rate: Proceed as
262 directed in the operating conditions in the preparation 1).

263 Column: A stainless steel column 4.6 mm in inside di-
264 ameter and 25 cm in length, packed with carbamoyl group
265 bound silica gel for liquid chromatography (5 μm in parti-
266 cle diameter).

267 Mobile phase: A mixture of acetonitrile, water and phos-
268 phoric acid (400:100:1).

269 *System suitability*—

270 System performance and system repeatability: Proceed
271 as directed in the system suitability in the preparation 1).

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

273 **Add the following to 9.41 Reagents,**

274 **Test Solutions:**

275 **Anemarrhena Rhizome** [Same as the namesake mon-
276 ograph]

277 **Mangiferin for assay** C₁₉H₁₈NO₁₁ Yellow crystals
278 or crystalline powder. Practically insoluble in water and in
279 ethanol (99.5). It is used after correction based on the con-
280 tent obtained in the Assay.

281 *Identification*—Proceed as directed in the Assay: it ex-
282 hibits a multiplet signal equivalent to one proton around δ
283 3.15 ppm, a multiplet signal equivalent to one proton
284 around δ 3.19 ppm, a multiplet signal equivalent to one pro-
285 ton around δ 3.22 ppm, a multiplet signal equivalent to one
286 proton around δ 3.43 ppm, a doublet-like signal equivalent
287 to one proton around δ 3.71 ppm, a triplet-like signal equiv-
288 alent to one proton around δ 4.07 ppm, a doublet signal
289 equivalent to one proton around δ 4.61 ppm, a singlet signal
290 equivalent to one proton around δ 6.40 ppm, a singlet signal
291 equivalent to one proton around δ 6.89 ppm and a singlet
292 signal equivalent to one proton around δ 7.40 ppm.

293 *Unity of peak*—Dissolve 1 mg of mangiferin for assay in
294 20 mL of diluted methanol (1 in 2), and use this solution as
295 the sample solution. Perform the test with 10 μL of the sam-
296 ple solution as directed under Liquid Chromatography
297 <2.01> according to the following conditions, and compare
298 the absorption spectra of at least 3 points including the top
299 of mangiferin peak and around the two middle peak heights

300 of before and after the top: no difference in form is ob-
301 served among their spectra.

302 Operating conditions

303 Column, column temperature, mobile phase, and flow
304 rate: Proceed as directed in the operating conditions in the
305 Assay (1) under Byakkokaninjinto Extract.

306 Detector: A photodiode array detector (wavelength: 367
307 nm, measuring range of spectrum: 220 – 400 nm).

308 System suitability

309 System performance: When the procedure is run with 10
310 μL of the sample solution under the above operating con-
311 ditions, the number of theoretical plates and the symmetry
312 factor of the peak of mangiferin are not less than 5000 and
313 not more than 1.5, respectively.

314 Assay—Weigh accurately 5 mg of mangiferin for assay
315 and 1mg of DSS- d_6 for nuclear magnetic resonance spec-
316 troscopy using an ultramicrobalance, dissolve them in 1 mL
317 of deuterated dimethylsulfoxide for nuclear magnetic reso-
318 nance spectroscopy, and use this solution as the sample so-
319 lution. Transfer the sample solution into an NMR tube 5
320 mm in outer diameter, measure ^1H -NMR as directed under
321 Nuclear Magnetic Resonance Spectroscopy <2.21> and
322 Crude Drugs Test <5.01> according to the following condi-
323 tions, using DSS- d_6 for nuclear magnetic resonance spec-
324 troscopy as the internal reference compound. Calculate the
325 resonance intensities, A_1 (equivalent to 1 proton), A_2
326 (equivalent to 1 proton) and A_3 (equivalent to 1 proton), of
327 the signals around δ 6.40 ppm, δ 6.89 ppm and δ 7.40 ppm
328 assuming the signal of the internal reference compound as
329 δ 0 ppm.

330 Amount (%) of mangiferin ($\text{C}_{19}\text{H}_{18}\text{O}_{11}$)

$$331 = M_S \times I \times P / (M \times N) \times 1.8824$$

332 M : Amount (mg) of mangiferin for assay taken

333 M_S : Amount (mg) of DSS- d_6 for nuclear magnetic reso-
334 nance spectroscopy taken

335 I : Sum of the signal resonance intensities, A_1 , A_2 and A_3 ,
336 based on the signal resonance intensity of DSS- d_6 for
337 nuclear magnetic resonance spectroscopy as 9.000

338 N : Sum of the numbers of the proton derived from A_1 , A_2
339 and A_3

340 P : Purity (%) of DSS- d_6 for nuclear magnetic resonance
341 spectroscopy

342 Operating conditions

343 Apparatus: A nuclear magnetic resonance spectrometer
344 having ^1H resonance frequency of not less than 400 MHz.

345 Target nucleus: ^1H .

346 Digital resolution: 0.25 Hz or lower.

347 Measuring spectrum range: 20 ppm or upper, including
348 between -5 ppm and 15 ppm.

349 Spinning: off.

350 Pulse angle: 90° .

351 ^{13}C decoupling: on.

352 Delay time: Repeating pulse waiting time not less than
353 60 seconds.

354 Integrating times: 8 or more times.

355 Dummy scanning: 2 or more times.

356 Measuring temperature: A constant temperature between
357 20°C and 30°C .

358 System suitability

359 Test for required detectability: When the procedure is
360 run with the sample solution under the above operating
361 conditions, the SN ratio of the signal around δ 6.40 ppm, δ
362 6.89 ppm and δ 7.40 ppm are not less than 100.

363 System performance: When the procedure is run with the
364 sample solution under the above operating conditions, the
365 signals around δ 6.40 ppm, δ 6.89 ppm and δ 7.40 ppm are
366 not overlapped with any signal of obvious foreign sub-
367 stances. Furthermore, the ratios of the resonance intensities,
368 A_1/A_2 , A_1/A_3 and A_2/A_3 , are between 0.99 and 1.01.

369 System repeatability: When the test is repeated 6 times
370 with the sample solution under the above operating condi-
371 tions, the relative standard deviations of the ratios of the
372 resonance intensities, A_1 , A_2 and A_3 to that of the internal
373 reference compound are not more than 1.0%.

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