

1 Unseiin Extract

2 温清飲エキス

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4 Unseiin Extract contains not less than 24 mg and
5 not more than 72 mg (for preparation prescribed 3 g
6 of Peony Root), or not less than 32 mg and not more
7 than 96 mg (for preparation prescribed 4 g of Peony
8 Root) of paeoniflorin (C₂₃H₂₈O₁₁:480.46), not less
9 than 39 mg and not more than 117 mg (for preparation
10 prescribed 1.5 g of Scutellaria Root), or not less than
11 78 mg and not more than 234 mg (for preparation pre-
12 scribed 3 g of Scutellaria Root) of baicalin
13 (C₂₁H₁₈O₁₁: 446.36), and not less than 20 mg and not
14 more than 60 mg of berberine [as berberine chloride
15 (C₂₀H₁₈ClNO₄: 371.81)], per extract prepared with
16 the amount specified in the Method of preparation.

17 Method of preparation

18

	1)	2)	3)
Japanese Angelica Root	4 g	4 g	3 g
Rehmannia Root	4 g	4 g	3 g
Peony Root	3 g	4 g	3 g
Cnidium Rhizome	3 g	4 g	3 g
Scutellaria Root	3 g	3 g	1.5 g
Gardenia Fruit	2 g	2 g	1.5 g
Coptis Rhizome	1.5 g	1.5 g	1.5 g
Phellodendron Bark	1.5 g	1.5 g	1.5 g

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20 Prepare a dry extract or viscous extract as directed under
21 Extracts, according to the prescription 1) to 3), using the
22 crude drugs shown above.

23 **Description** Unseiin Extract occurs as a yellow-brown to
24 very dark brown powder or blackish brown viscous extract.
25 It has a slight odor, and has a slightly sweet taste at first,
26 followed by a pungent taste.

27 **Identification** (1) To 1.0 g of the dry extract (or 3.0 g
28 of the viscous extract) add 15 mL of water and 5 mL of 0.1
29 mol/L hydrochloric acid, shake, then add 25 mL of diethyl
30 ether, and shake. Separate the diethyl ether layer, evaporate
31 the solvent under reduced pressure, add 2 mL of diethyl
32 ether to the residue, and use this solution as the sample so-
33 lution. Separately, use (Z)-ligustilide TS for thin-layer
34 chromatography as the standard solution. Perform the test
35 with these solutions as directed under Thin layer Chroma-
36 tography <2.03>. Spot 10 μL each of the sample solution
37 standard solution on a plate of silica gel for thin-layer chro-
38 matography. Develop the plate with a mixture of butyl ac-
39 etate and hexane (2:1) to a distance of about 7 cm, and air-
40 dry the plate. Examine under ultraviolet light (main wave-
41 length: 365 nm): one of the several spots obtained from the
42 sample solution has the same color tone and R_f value with
43 the bluish-white fluorescent spot from the standard solution
44 (Japanese Angelica Root; Cnidium Rhizome).

45 (2) To 0.5 g of the dry extract (or 1.5 g of the viscous
46 extract) add 10 mL of methanol, shake, centrifuge, and use
47 the supernatant liquid as the sample solution. Separately,
48 dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-
49 layer chromatography in 1 mL of methanol, and use this
50 solution as the standard solution. Perform the test with
51 these solutions as directed under Thin-layer Chromatog-
52 raphy <2.03>. Spot 5 μL each of the sample solution and
53 standard solution on a plate of silica gel for thin-layer chro-
54 matography. Develop the plate with a mixture of ethyl ac-
55 etate, methanol and water (20:3:2) to a distance of about 7
56 cm, and air-dry the plate. Spray evenly 4-methoxybenzal-
57 dehyde-sulfuric acid TS on the plate, and heat at 105°C for
58 1 minute: one of the several spots obtained from the sample
59 solution has the same color tone and R_f value with the red-
60 purple to purple spot from the standard solution (Peony
61 Root).

62 (3) To 1.0 g of the dry extract (or 3.0 g of the viscous
63 extract) add 10 mL of water, shake, then add 25 mL of di-
64 ethyl ether, and shake. Separate the diethyl ether layer,
65 evaporate the solvent under reduced pressure, then add 2
66 mL of diethyl ether to the residue, and use this solution as
67 the sample solution. Separately, dissolve 1 mg of wogonin
68 for thin-layer chromatography in 1 mL of methanol, and
69 use this solution as the standard solution. Perform the test
70 with these solutions as directed under Thin-layer Chroma-
71 tography <2.03>. Spot 20 μL of the sample solution and 5
72 μL of the standard solution on a plate of silica gel for thin-
73 layer chromatography. Develop the plate with a mixture of
74 ethyl acetate, hexane and acetic acid (100) (10:10:1) to a
75 distance of about 7 cm, and air-dry the plate. Spray evenly
76 iron (III) chloride-methanol TS on the plate: one of the sev-
77 eral spots obtained from the sample solution has the same
78 color tone and R_f value with the yellow-brown to grayish
79 brown spot from the standard solution (Scutellaria Root).

80 (4) To 0.5 g of the dry extract (or 1.5 g of the viscous
81 extract) add 10 mL of methanol, shake, centrifuge, and use
82 the supernatant liquid as the sample solution. Separately,
83 dissolve 1 mg of geniposide for thin-layer chromatography
84 in 1 mL of methanol, and use this solution as the standard
85 solution. Perform the test with these solutions as directed
86 under Thin-layer Chromatography <2.03>. Spot 5 μL each
87 of the sample solution and standard solution on a plate of
88 silica gel for thin-layer chromatography. Develop the plate
89 with a mixture of ethyl acetate, methanol and water
90 (20:3:2) to a distance of about 7 cm, and air-dry the plate.
91 Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on
92 the plate, and heat at 105°C for 1 minute: one of the several
93 spots obtained from the sample solution has the same color
94 tone and R_f value with the purple to dark purple spot from
95 the standard solution (Gardenia Fruit).

96 (5) To 0.5 g of the dry extract (or 1.5 g of the viscous
97 extract) add 10 mL of methanol, shake, centrifuge, and use
98 the supernatant liquid as the sample solution. Separately,
99 dissolve 1 mg of coptisine chloride for thin-layer chroma-
100 tography in 5 mL of methanol, and use this solution as the
101 standard solution. Perform the test with these solutions as
102 directed under Thin-layer Chromatography <2.03>. Spot 2
103 μL each of the sample solution and standard solution on a
104 plate of silica gel for thin-layer chromatography. Develop
105 the plate with a mixture of ethyl acetate, ammonia solution
106 (28) and methanol (15:1:1) to a distance of about 7 cm, and
107 air-dry the plate. Examine under ultraviolet light (main
108 wavelength: 365 nm): one of the several spots obtained
109 from the sample solution has the same color tone and R_f
110 value with the yellow fluorescent spot from the standard
111 solution (Coptis Rhizome).

112 (6) To 1.0 g of dry extract (or 3.0 g of the viscous ex-
113 tract) add 10 mL of water, shake, then add 25 mL of diethyl
114 ether, and shake. Separate the diethyl ether layer, evaporate
115 the solvent under reduced pressure, then add 2 mL of di-
116 ethyl ether to the residue, and use this solution as the sam-
117 ple solution. Separately, dissolve 1 mg of limonin for thin-
118 layer chromatography in 1 mL of methanol, and use this
119 solution as the standard solution. Perform the test with
120 these solutions as directed under Thin-layer Chromatog-
121 raphy <2.03>. Spot 10 μL of the sample solution and 5 μL
122 of the standard solution on a plate of silica gel for thin-layer
123 chromatography. Develop the plate with a mixture of ethyl
124 acetate, hexane and acetic acid (100) (10:5:1) to a distance
125 of about 7 cm, and air-dry the plate. Spray evenly vanillin-
126 sulfuric acid-ethanol TS for spraying on the plate, heat at
127 105°C for 5 minutes, and allow to cool: one of the several
128 spots obtained from the sample solution has the same color
129 tone and R_f value with the purple to dark purple spot from
130 the standard solution (Phellodendron Bark).

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

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

140 **Loss on drying <2.41>** The dry extract: Not more than
141 10.0% (1 g, 105°C, 5 hours).

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

144 **Total ash <5.01>** Not more than 9.0%, calculated on the
145 dried basis.

146 **Assay (1)** Paeoniflorin—Weigh accurately about 0.5 g
147 of the dry extract (or an amount of the viscous extract,
148 equivalent to about 0.5 g of the dried substance), add ex-
149 actly 50 mL of diluted methanol (1 in 2), shake for 15
150 minutes, and filter. Pipet 5 mL of the filtrate, flow through
151 in a column packed with 2 g of polyamide for column chro-
152 matography, elute with 20 mL of water, then add 1 mL of
153 acetic acid (100) to the eluate, add water to make exactly
154 25 mL, and use this solution as the sample solution. Sepa-
155 rately, weigh accurately about 10 mg of Paeoniflorin RS
156 (separately determine the water <2.48> by coulometric ti-
157 tration, using 10 mg), and dissolve in diluted methanol (1
158 in 2) to make exactly 100 mL. Pipet 5 mL of this solution,
159 add diluted methanol (1 in 2) to make exactly 20 mL, and
160 use this solution as the standard solution. Perform the test
161 with exactly 10 μL each of the sample solution and stand-
162 ard solution as directed under Liquid Chromatography
163 <2.01> according to the following conditions, and deter-
164 mine the peak areas, A_T and A_S , of paeoniflorin in each so-
165 lution.

$$166 \quad \text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}\text{)} \\ 167 \quad = M_S \times A_T / A_S \times 5 / 8$$

168 M_S : Amount (mg) of Paeoniflorin RS taken, calculated
169 on the anhydrous basis

170 **Operating conditions—**

171 **Detector:** An ultraviolet absorption photometer (wave-
172 length: 232 nm).

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

177 **Column temperature:** A constant temperature of about
178 20°C.

179 **Mobile phase:** A mixture of water, acetonitrile and phos-
180 phoric acid (850:150:1).

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

182 **System suitability—**

183 **System performance:** Dissolve 1 mg each of Paeoniflorin
184 RS and albiflorin in diluted methanol (1 in 2) to make 10
185 mL. When the procedure is run with 10 μL of this solution
186 under the above operating conditions, albiflorin and pae-
187 oniflorin are eluted in this order with the resolution be-
188 tween these peaks being not less than 2.5.

189 **System repeatability:** When the test is repeated 6 times
190 with 10 μL of the standard solution under the above oper-
191 ating conditions, the relative standard deviation of the peak
192 area of paeoniflorin is not more than 1.5%.

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

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

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

209 $=M_S \times A_T/A_S \times 1/4$

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

212 *Operating conditions—*

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

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

219 Column temperature: A constant temperature of about
 220 40°C.

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

223 Flow rate: 1.0 mL per minute.

224 *System suitability—*

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

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

234 (3) Berberine—Weigh accurately about 0.2 g of the
 235 dry extract (or an amount of the viscous extract, equivalent
 236 to about 0.2 g of dried substance), add exactly 50 mL of the
 237 mobile phase, shake for 15 minutes, then filter, and use the
 238 filtrate as the sample solution. Separately, weigh accurately
 239 about 10 mg of Berberine Chloride RS (separately deter-
 240 mine the water <2.48> in the same manner as Berberine
 241 Chloride Hydrate), dissolve in the mobile phase to make
 242 exactly 100 mL, and use this solution as the standard solu-
 243 tion. Perform the test with exactly 10 μ L each of the sample
 244 solution and standard solution as directed under Liquid
 245 Chromatography <2.01> according to the following condi-
 246 tions, and determine the peak areas, A_T and A_S , of berberine
 247 in each solution.

248 Amount (mg) of berberine chloride ($C_{20}H_{18}ClNO_4$)
 249 $=M_S \times A_T/A_S \times 1/2$

250 M_S : Amount (mg) of Berberine Chloride RS taken, cal-
 251 culated on the anhydrous basis

252 *Operating conditions—*

253 Detector: An ultraviolet absorption photometer (wave-
 254 length: 345 nm).

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

259 Column temperature: A constant temperature of about
 260 30°C.

261 Mobile phase: Dissolve 3.4 g of potassium dihydrogen
 262 phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of
 263 a mixture of water and acetonitrile (1:1).

264 Flow rate: 1.0 mL per minute.

265 *System suitability—*

266 System performance: Dissolve 1 mg each of Berberine
 267 Chloride RS and palmatine chloride in the mobile phase to
 268 make 10 mL. When the procedure is run with 10 μ L of this
 269 solution under the above operating conditions, palmatine
 270 and berberine are eluted in this order with the resolution
 271 between these peaks being not less than 1.5.

272 System repeatability: When the test is repeated 6 times
 273 with 10 μ L of the standard solution under the above oper-
 274 ating conditions, the relative standard deviation of the peak
 275 area of berberine is not more than 1.5%.

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