Ganoderma lucidum Fruiting Body Dry Extract

Final Authorized Version 1.0

*Ganoderma lucidum* Fruiting Body Dry Extract

**DEFINITION**
The article is prepared from the dried fruiting bodies of *Ganoderma lucidum* (Curtis: Fr.) P. Karst. (Family Ganodermataceae) by sequential extraction, first with alcohol and then with water, and combining these extractions. It contains NLT 90.0% and NMT 110.0% of the labeled amount of triterpene acids, calculated as the sum of ganoderenic acid C, ganoderic acid C₂, ganoderic acid G, ganoderenic acid B, ganoderic acid B, ganoderic acid A, ganoderic acid H, ganoderenic acid D, ganoderic acid D, and ganoderic acid F on the dried basis. It may contain suitable added substances as carriers.

**POTENTIAL CONFOUNGING MATERIALS**
- Amauroderma rude
- Amauroderma rugosum
- Coriolus versicolor
- Phellinus baumii
- Related *Ganoderma* species including *G. sinense* (*G. japonicum*), *G. atrum*, *G. tsugae*, *G. applanatum*, *G. ungulatum*, *G. capense*, and *G. resinaceum*

**CONSTITUENTS OF INTEREST**
- **Triterpene acids:** Ganoderenic acid C, ganoderic acid C₂, ganoderic acid G, ganoderenic acid B, ganoderic acid B, ganoderic acid A, ganoderic acid H, ganoderenic acid D, ganoderic acid D, and ganoderic acid F
- **Polysaccharides**

**IDENTIFICATION**

**A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution A:** 1.0 mg/mL of USP Ganoderic Acid A RS in alcohol

**Standard solution B:** 0.3 mg/mL of USP Ergosterol RS in alcohol

**Standard solution C:** 50 mg/mL of USP *Ganoderma lucidum* Fruiting Body Dry Extract RS in alcohol. Sonicate for about 10 min, centrifuge, and use the supernatant.

**Sample solution:** 50 mg/mL of *Ganoderma lucidum* Fruiting Body Dry Extract in alcohol. Sonicate for about 10 min, centrifuge, and use the supernatant.

**Chromatographic system**

(See Chromatography <621> [4], Thin-Layer Chromatography.)

**Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates). Pre-develop the plate in methanol and dry at 105° for 30 min.
Application volume: 2 µL each of Standard solution A and Standard solution B, and 4 µL each of Standard solution C and Sample solution, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: Room temperature and not to exceed 30°

Developing solvent system: Toluene, ethyl formate, and formic acid (5: 5: 0.2)

Developing distance: 7 cm

Derivatization reagent: A solution of 10% sulfuric acid in alcohol. [Note—Prepare fresh. Keep alcohol cold over ice, carefully and gradually add sulfuric acid.]

Analysis

Samples: Standard solution A, Standard solution B, Standard solution C, and Sample solution

Apply the Samples as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent, and heat for about 5 min at 105°–110°. Immediately examine under UV light at 366 nm and visible light.

System suitability: Under UV light at 366 nm, the chromatogram of Standard solution C exhibits, in the lower-third section, six characteristic colorful bands in the following order of increasing \( R_f \): an orange band; another orange band due to ganoderic acid C₂; a green to yellowish-green band due to ganoderic acid A and ganoderenic acid A at an \( R_f \) corresponding to the band due to ganoderic acid A in the chromatogram of Standard solution A; a yellow band due to ganoderic acid G, ganoderenic acid B, and ganoderic acid B; an orange band due to ganoderic acid H; and a bluish-green band due to ganoderenic acid D and ganoderic acid D. Standard solution C also exhibits an orange band at about the middle of the chromatogram and a bluish fluorescent band in the upper-third section at an \( R_f \) corresponding to the band due to ergosterol in the chromatogram of Standard solution B.

Under visible light, the chromatogram of Standard solution C exhibits a bluish-violet band corresponding in \( R_f \) to the ganoderic acid A band in the chromatogram of Standard solution A; two reddish-violet bands, one at an \( R_f \) below that of ganoderic acid A band (due to ganoderic acid C₂) and another above (due to ganoderic acid G, ganoderenic acid B, and ganoderic acid B); a bluish-violet band at about the middle of the chromatogram; and a brownish-violet band at about two-thirds corresponding in color and \( R_f \) to the ergosterol band in the chromatogram of Standard solution C.

Acceptance criteria: Under UV light at 366 nm, the chromatogram of the Sample solution exhibits six bands corresponding in color and \( R_f \) to similar bands in the chromatogram of Standard solution C (distinction from Amauroderma rude, Amauroderma rugosum, Coriolus versicolor, Ganoderma sinense, G. applanatum, G. tsugae, G. ungulatum, G. capense, G. resinaceum, and Phellinus baumii). These six bands appear in the following order of increasing \( R_f \): two orange bands; a green to yellowish-green band at an \( R_f \) corresponding to the band due to ganoderic acid A in the chromatogram of Standard solution A; a yellow band; an orange band; and a bluish-green band. The Sample solution also exhibits an orange band at about the middle of the chromatogram, and a bluish fluorescent band in the upper-third section at an \( R_f \) corresponding to the band due to ergosterol in the chromatogram of Standard solution B.

Under visible light, the Sample solution exhibits a bluish-violet band corresponding in \( R_f \) to the ganoderic acid A band in the chromatogram of Standard solution A; two reddish-violet bands, one above and the other below the ganoderic acid A band; a bluish-violet band at about the middle of the chromatogram; and two violet bands in the upper-third section, one at an \( R_f \) corresponding to that of the ergosterol band in the chromatogram of Standard solution B and one above the ergosterol band.

• B. LIQUID CHROMATOGRAPHY PROFILE OF TRITERPENE ACIDS

Analysis: Proceed as directed in the Assay for Content of Triterpene Acids.

Acceptance criteria: The Sample solution chromatogram exhibits peaks at the retention times corresponding to ganoderenic acid C, ganoderic acid C₂, ganoderic acid G, ganoderenic acid B, ganoderic
acid B, ganoderenic acid A, ganoderic acid A, ganoderic acid H, ganoderenic acid D, ganoderic acid D, and ganoderic acid F in the chromatogram of Standard solution B.

**ASSAY**

**CONTENT OF TRITERPENE ACIDS**

**Solution A:** 0.075% Phosphoric acid in water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1. [Note—Adjust the gradient program in Table 1 to maintain the Mobile phase composition in the second step at 73.5% Solution A until the complete elution of the ganoderic acid A peak. Then change the Mobile phase composition in the third step over 18 min to 61.5% Solution A.]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>73.5</td>
<td>26.5</td>
</tr>
<tr>
<td>34</td>
<td>73.5</td>
<td>26.5</td>
</tr>
<tr>
<td>52</td>
<td>61.5</td>
<td>38.5</td>
</tr>
<tr>
<td>54</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>55</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>55.5</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

**Standard solution A:** 0.1 mg/mL of USP Ganoderic Acid A RS in methanol

**Standard solution B:** Sonicate 200 mg of USP Ganoderma lucidum Fruiting Body Dry Extract RS in 25 mL of alcohol and centrifuge. Mix 2 mL of the supernatant with 18 mL of water, transfer to a solid phase extraction column containing L1 packing with a sorbent mass-to-column volume ratio of 200 mg/5 mL. Condition the column as described next and elute at the rate of 1 drop/s. Wash the column with 3 mL of water and discard the washing. Elute the column with methanol into a 2-mL volumetric flask, continue to collect 2.0 mL, and mix. Before injection, pass through a nylon filter having a 0.2-µm pore size, and discard the first portion of the filtrate.

[Note—Initially condition the solid phase extraction column with 5 mL of methanol and then 3 mL of water. Do not allow the column to dry.]

**Sample solution:** Use 200 mg of Ganoderma lucidum Fruiting Body Dry Extract and proceed as directed for Standard solution B.

**Chromatographic system**

(See Chromatography <621> [1], System Suitability.)

**Mode:** UHPLC

**Detector:** UV 257 nm

**Column:** 2.1-mm × 15-cm; 1.8-µm packing L1 (similar to ACQUITY UPLC HSS T3, Zorbax SB C-18, and Eclipse Plus C-18)

**Column temperature:** 25 ± 1°

**Flow rate:** 0.4 mL/min

**Injection volume:** 5 µL

**System suitability**
**Samples:** Standard solution A and Standard solution B

**Suitability requirements**

**Chromatogram similarity:** The chromatogram from Standard solution B is similar to the reference chromatogram provided with the lot of USP *Ganoderma lucidum* Fruiting Body Dry Extract RS being used.

**Resolution:** NLT 1.0 between ganoderic acid A and ganoderic acid H peaks, Standard solution B

**Tailing factor:** NMT 2.0 for the ganoderic acid A peak, Standard solution A

**Relative standard deviation:** NMT 2.0% determined from ganoderic acid A peak in repeated injections, Standard solution A

**Analysis**

**Samples:** Standard solution A, Standard solution B, and Sample solution. [Note—Sample solution is stable for 24 h at room temperature.]

Using the chromatograms of Standard solution A, Standard solution B, and the reference chromatogram provided with the lot of USP *Ganoderma lucidum* Fruiting Body Dry Extract RS being used, identify the retention times of the peaks corresponding to ganoderenic acid C, ganoderic acid C₂, ganoderic acid G, ganoderenic acid B, ganoderic acid B, ganoderic acid A, ganoderic acid H, ganoderenic acid D, ganoderic acid D, and ganoderic acid F. The approximate relative retention times, relative to ganoderic acid A, are provided in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganoderenic acid C</td>
<td>0.36</td>
<td>0.51</td>
</tr>
<tr>
<td>Ganoderic acid C₂</td>
<td>0.42</td>
<td>1.05</td>
</tr>
<tr>
<td>Ganoderic acid G</td>
<td>0.56</td>
<td>1.18</td>
</tr>
<tr>
<td>Ganoderenic acid B</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>Ganoderic acid B</td>
<td>0.66</td>
<td>1.10</td>
</tr>
<tr>
<td>Ganoderic acid A</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Ganoderic acid H</td>
<td>1.05</td>
<td>1.54</td>
</tr>
<tr>
<td>Ganoderenic acid D</td>
<td>1.25</td>
<td>0.51</td>
</tr>
<tr>
<td>Ganoderic acid D</td>
<td>1.33</td>
<td>1.08</td>
</tr>
<tr>
<td>Ganoderic acid F</td>
<td>1.54</td>
<td>1.45</td>
</tr>
</tbody>
</table>
Separately calculate the percentage of each of the triterpene acids in the portion of *Ganoderma lucidum* Fruiting Body Dry Extract taken.

\[
\text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_S}{C_u} \right) \times F \times 100
\]

- \( r_u \) = peak response of the analyte from the *Sample solution*
- \( r_s \) = peak response of ganoderic acid A from *Standard solution A*
- \( C_s \) = concentration of ganoderic acid A in *Standard solution A* (mg/mL)
- \( C_u \) = concentration of *Ganoderma lucidum* Fruiting Body Dry Extract in the *Sample solution* (mg/mL)
- \( F \) = conversion factors for the analytes as provided in Table 2

*Note*—Incorporate a factor in the calculation to account for the aliquot taken compared to the volume of the *Sample solution*.

Calculate the content of triterpene acids as the sum of the percentages of the individual triterpene acids.

Calculate the percentage of the labeled amount of triterpene acids in the Extract:

\[
\text{Result} = \left( \frac{P}{L} \right) \times 100
\]

- \( P \) = content of triterpene acids as determined above (%)
- \( L \) = labeled amount of triterpene acids (%)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of triterpene acids on the dried basis

**CONTAMINANTS**

  - **Acceptance criteria**
    - Arsenic: NMT 3.0 µg/g
    - Cadmium: NMT 0.5 µg/g
    - Lead: NMT 5.0 µg/g
    - Mercury: NMT 0.2 µg/g

- **Articles of Botanical Origin [7]**, *General Method for Pesticide Residues Analysis <561>*: Meets the requirements

- **Microbial Enumeration Tests <61> [8]**: The total aerobic bacterial count does not exceed \( 10^4 \) cfu/g and the total combined molds and yeasts count does not exceed \( 10^2 \) cfu/g.

- **Tests for Specified Microorganisms <62> [9]**: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**SPECIFIC TESTS**

- **Loss on Drying <731> [10]**
  - **Sample:** 1.0 g of *Ganoderma lucidum* Fruiting Body Dry Extract
  - **Analysis:** Dry the *Sample* at 105° for 2 h.
  - **Acceptance criteria:** NMT 7.0%

**ADDITIONAL REQUIREMENTS**
• **Packaging and Storage**: Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.

• **Labeling**: The label states the Latin binomial and, following the official name, the parts of the plant from which the article was derived. It meets other labeling requirements under *Botanical Extracts <565>* [11].

• **USP Reference Standards <11>** [12]
  
  USP Ergosterol RS [2]
  
  USP Ganoderic Acid A RS [1]
  
  USP *Ganoderma lucidum* Fruiting Body Dry Extract RS [3]

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