Ganoderma lucidum Fruiting Body Powder

Final Authorized Version 1.0

*Ganoderma lucidum* Fruiting Body Powder

**DEFINITION**
The article consists of the dried fruiting bodies of *Ganoderma lucidum* (Curtis: Fr.) P. Karst. (Family Ganodermataceae) reduced to a powder or very fine powder. It contains NLT 0.3% of triterpene acid, 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.

**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. BOTANICAL CHARACTERISTICS**
  - **Macroscopic:** Light brown coarse powder
  - **Microscopic:** Fragments of the hyphae of the cap with the densely packed underlying plectenchyma, fragments of hyphae showing palisade arrangement and rounded ends, fragments of binding hyphae, dark brown fragments of the tubes, basidiospores, and basidia

- **B. THIN-LAYER CHROMATOGRAPHY**
  - **Standard solution A:** 1.0 mg/mL of *USP Ganoderic Acid A RS* [1] in alcohol
  - **Standard solution B:** 0.3 mg/mL of *USP Ergosterol RS* [2] in alcohol
  - **Standard solution C:** 50 mg/mL of *USP Ganoderma lucidum Fruiting Body Dry Extract RS* [3] in alcohol.
    Sonicate for about 10 min, centrifuge, and use the supernatant.
  - **Sample solution:** Sonicate about 1.0 g of *Ganoderma lucidum* Fruiting Body Powder in 50 mL of alcohol for 15 min, centrifuge, and evaporate the supernatant to dryness under reduced pressure at a
temperature not to exceed 50°. Dissolve the residue in 2.0 mL of alcohol, centrifuge, and use the supernatant.

**Chromatographic system**
(See *Chromatography* [621], 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 Rf: 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 Rf 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 Rf corresponding to the band due to ergosterol in the chromatogram of Standard solution B.

Under visible light, Standard solution C exhibits a bluish-violet band corresponding in Rf to the ganoderic acid A band in the chromatogram of Standard solution A; two reddish-violet bands, one at an Rf 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 Rf 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 Rf 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 Rf: two orange bands; a green to yellowish-green band at an Rf 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 Rf 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 Rf 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.

**C. 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.

**D. Constituting Monosaccharides of Water-Soluble Polysaccharides**

**Analysis:** Proceed as directed in the Specific Test for Content of Water-Soluble Polysaccharides.

**Acceptance criteria:** The Sample solution chromatogram exhibits peaks at the retention times corresponding to mannose, glucuronic acid, glucose, galactose, and fucose in the chromatogram of Standard solution B. The most intense peak is that of glucose; the peaks due to mannose and galactose are of similar intensity; glucuronic acid is of lesser intensity; fucose is a minor peak.

**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 1

<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 [1] in methanol

**Standard solution B:** Sonicate 200 mg of USP Ganoderma lucidum Fruiting Body Dry Extract RS [3] 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 per second. 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 stock solution:** Transfer about 2.0 g of Ganoderma lucidum Fruiting Body Powder to a 200-mL
round-bottom flask, add 75 mL of alcohol, reflux for 45 min, cool, and filter. Rinse the round-bottom flask twice with 10 mL of alcohol, filter, and add the washings to the filtrate. Evaporate the filtrate under reduced pressure to dryness, and dissolve the residue in NMT 25 mL of alcohol. Transfer the solution to a 25-mL volumetric flask, dilute with alcohol to volume, and mix.

**Sample solution:** Add 2 mL of *Sample stock solution* to 18 mL of water, and mix well. Transfer the solution 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.]

**Chromatographic system**

(See *Chromatography* &lt;621&gt;[4], *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* [3] 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* [3] 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>Triterpene Acid</td>
<td>Concentration</td>
<td>Ratio</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------</td>
<td>-------</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 Powder taken.

Result = \( \frac{r_U}{r_S} \times C_S \times \frac{V}{W} \times F \times 100 \)

- \( r_U \) = 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)
- \( V \) = volume of the *Sample stock solution* (mL)
- \( W \) = weight of *Ganoderma lucidum* Fruiting Body Powder taken to prepare the *Sample solution* (mg)
- \( F \) = 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.

**Acceptance criteria:** NLT 0.3% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures <233>**

**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** \[6\], *General Method for Pesticide Residues Analysis* <561>: Meets the requirements

• **MICROBIAL ENUMERATION TESTS** <61>: The total aerobic bacterial count does not exceed $10^5$ cfu/g, the total combined molds and yeasts count does not exceed $10^3$ cfu/g, and the bile-tolerant Gram-negative bacteria does not exceed $10^1$ cfu/g.

• **TESTS FOR SPECIFIED MICROORGANISMS** <62>: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**SPECIFIC TESTS**

• **CONTENT OF WATER-SOLUBLE POLYSACCHARIDES**

  **Solution A**: 0.05 M phosphate buffer (pH 6.0)
  **Solution B**: Acetonitrile
  **Mobile phase**: See Table 3.

  **Table 3**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>82.5</td>
<td>17.5</td>
</tr>
<tr>
<td>55</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>60</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>61</td>
<td>84</td>
<td>16</td>
</tr>
</tbody>
</table>

  **Reagent**: 0.1 M methanolic solution of 1-phenyl-3-methyl-5-pyrazolone
  **Internal standard solution**: 0.5 mg/mL of D-lyxose in water
  **Standard solution**: Composite solution containing 0.20 mg/mL each of USP Mannose RS \[9\], USP D-Glucuronic Acid RS \[10\], and USP Galactose RS \[11\], 2.0 mg/mL of USP Dextrose RS \[12\], and 0.1 mg/mL of USP L-Fucose RS \[13\] in water.
  **Sample solution**: Transfer 2.0 g of *Ganoderma lucidum* Fruiting Body Powder to a round-bottom flask, add 60 mL of water, allow to stand for 1 h, heat under reflux for 4 h, and filter immediately. Transfer the residue and the filter paper to the same flask, add 60 mL of water, heat under reflux for 3 h, and filter immediately. Rinse the flask three times with 5 mL of water and filter. Transfer the combined filtrates and washings to a 250-mL beaker, evaporate on a water bath to dryness. Dissolve the residue in 5.0 mL of water, add 75 mL of alcohol, mix well, allow to stand at 4° for 12 h, and centrifuge at 4000 rpm for 30 min. Discard the supernatant and dry the precipitate. Dissolve the residue in hot water, quantitatively transfer the solution to a 10-mL volumetric flask, dilute with water to volume, and mix. Centrifuge the solution at 4000 rpm for 10 min. Transfer 0.25 mL of the supernatant, accurately measured, to a reaction vial and add 0.25 mL of 4 M trifluoroacetic acid. Seal the vial, heat at 110° for 4 h, cool to room temperature, add 0.50 mL of methanol, and evaporate to dryness at 60° under vacuum. Repeat the addition of 0.50 mL of methanol and evaporation three times. Add 0.125 mL of water to the residue.

  **Chromatographic system**
  (See *Chromatography* <621>, *System Suitability.*)
  **Mode**: HPLC
  **Detector**: UV 250 nm
  **Column**: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Zorbax XDB C18)
Column temperature: 35 ± 1°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: Standard solution

Suitability requirements
Tailing factor: NMT 2.0 for the dextrose peak
Relative standard deviation: NMT 2.0% determined from dextrose peak in repeated injections

Analysis
Samples: Standard solution and Sample solution. [Note—Derivatized Standard solution and Sample solution are stable for 24 h at room temperature.]

Combine 0.125 mL of Standard solution or Sample solution with 0.125 mL of Internal standard solution, 0.3 mL of 0.15 M sodium hydroxide solution, and 0.50 mL of Reagent in a capped reaction vial. Seal the vial, heat at 70° for 30 min, and cool to room temperature. Add 0.3 mL of 0.15 M hydrochloric acid and 0.65 mL of water to the vial, mix well, and pass through a nylon filter of 0.45-µm or finer pore size.

Using the chromatograms of derivatized Standard solution, and the reference chromatogram provided with the lot of USP Ganoderma lucidum Fructing Body Dry Extract RS [3] being used, identify the retention times of the peaks with respect to dextrose: 0.48 for mannose, 0.58 for lyxose, 0.82 for D-glucuronic acid, and 1.09 for galactose.

Separately calculate the percentages of derivatized monosaccharides in the portion of Ganoderma lucidum Fructing Body Powder taken:

\[
\text{Result} = \left( \frac{R_u}{R_s} \right) \times A_s \times \left( \frac{F}{W} \right) \times 100
\]

\( R_u \) = peak response ratio of the relevant analyte to the internal standard from the Sample solution
\( R_s \) = peak response ratio of the relevant analyte to the internal standard from the Standard solution
\( A_s \) = amount of the relevant analyte in the aliquot of Standard solution subject to derivatization (mg)
\( F \) = dilution factor to account for the sample aliquot submitted to hydrolysis (0.25 mL) relative to the volume of the Sample solution (10 mL), 40
\( W \) = weight of Ganoderma lucidum Fructing Body Powder taken to prepare the Sample solution (mg)

Add the percentages of mannose, glucuronic acid, glucose, and galactose.

Acceptance criteria: NLT 0.7% on the dried basis

- Loss on Drying: NLT 0.7% on the dried basis

  Sample: 1.0 g of Ganoderma lucidum Fructing Body Powder

  Analysis: Dry the Sample at 105° for 4 h.

  Acceptance criteria: NMT 17.0%

- Articles of Botanical Origin, Alcohol-Soluble Extractives, Method 1: NMT 2.0%
- Articles of Botanical Origin, Water-Soluble Extractives, Method 1: NLT 3.0%
- Articles of Botanical Origin, Total Ash: NMT 4.0%

Analysis: 3.0 g of Ganoderma lucidum Fructing Body Powder

Acceptance criteria: NMT 4.0%

**ADDITIONAL REQUIREMENTS**

- Packaging and Storage: Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
• **LABELING:** The label states the Latin binomial and the parts of the plant from which the article was derived.

• **USP Reference Standards <11> [5]**
  - USP Dextrose RS [12]
  - USP Ergosterol RS [2]
  - USP L-Fucose RS [13]
  - USP Galactose RS [11]
  - USP Ganoderic Acid A RS [1]
  - USP *Ganoderma lucidum* Fruiting Body Dry Extract RS [3]
  - USP α-Glucuronic Acid RS [10]
  - USP Mannose RS [9]

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