Ganoderma Lucidum Fruiting Body

**DEFINITION**

Ganoderma Lucidum Fruiting Body consists of the dried fruiting body of Ganoderma lucidum (W. Curt.:Fr.) P. Karst. (Fam. Ganodermataceae). It contains NLT 0.3% of triterpenoic acids, calculated on the dried basis as a sum of ganoderic acids A, B, C, D, F, G, and H and ganoderenic acids B, C, and D.

**IDENTIFICATION**

Change to read:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>A. THIN-LAYER CHROMATOGRAPHY</strong></td>
<td></td>
</tr>
<tr>
<td>Standard solution A:</td>
<td>1.0 mg/mL of USP Ganoderic Acid A RS in alcohol</td>
</tr>
<tr>
<td>Standard solution B:</td>
<td>0.3 mg/mL of USP Ergosterol RS in alcohol</td>
</tr>
<tr>
<td>Standard solution C:</td>
<td>50 mg/mL of USP Ganoderma Lucidum Fruiting Body Powdered Extract RS in alcohol</td>
</tr>
<tr>
<td>Sample solution:</td>
<td>Sonicate about 1 g of Ganoderma Lucidum Fruiting Body Powdered Extract RS in alcohol</td>
</tr>
</tbody>
</table>

**Derivatization**

Developing solvent system:

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

NOTEÐ The chromatogram of Ganoderma Lucidum Fruiting Body exhibits bands corresponding in color and relatively diffuse, corresponds to the ergosterol band in Standard solution A. Two or three light-brown bands are seen under white light in the upper third of the chromatogram of Standard solution C. [NOTE—The Standard solutions are stable for 72 h at room temperature.]

**Analysis**

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

Apply the samples as bands and dry in air. Develop in a saturated chamber, remove the plate, air-dry, treat with Derivatization reagent, and heat at 105°–110° for 5 min. Immediately examine under white light and under the long-wave UV light (365 nm).

**Acceptance criteria:** Under long-wave UV light (365 nm) and under white light, the chromatogram of the Sample solution exhibits bands corresponding in color and Rf to similar bands in the chromatogram of Standard solution C, at the Rf values listed for System suitability. Under white light, the chromatogram of the Sample solution exhibits an additional violet band above the ergosterol band. [NOTE—The Sample solution is stable for 72 h at room temperature.]

**B. HPLC**

Analysis: Proceed as directed in the test for Content of Triterpenoic Acids.

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

**C. HPLC**

Analysis: Proceed as directed in the test for Content of Water-Soluble Polysaccharides.

**Acceptance criteria:** The chromatogram of the Sample solution exhibits peaks at the retention times corresponding to the peaks due to mannose, glucuronic acid, dextrose, galactose, and L-fucose in the chromatogram of the Standard solution.

**COMPOSITION**

Change to read:

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>CONTENT OF TRITERPENOIC ACIDS</strong></td>
<td></td>
</tr>
<tr>
<td>Solution A: 0.075% phosphoric acid in water</td>
<td></td>
</tr>
<tr>
<td>Solution B: Acetonitrile</td>
<td></td>
</tr>
<tr>
<td>Mobile phase: See Table 1.</td>
<td></td>
</tr>
</tbody>
</table>

**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>80.0</td>
<td>20.0</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>53</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>58</td>
<td>80.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>
Ganoderma

[NOTE—Maintain the Mobile phase at 73.5% of Solution A for the period sufficient for complete elution of ganoderic acid A.]

**Standard solution A**: 0.1 mg/mL of USP Ganoderic Acid A RS (ERR 1-Dec-2014) in methanol. Sonicate to dissolve if necessary.

**Standard solution B**: Sonicate 40 mg of USP Ganoderma Lucidum Fruiting Body Powdered Extract RS in 5 mL of alcohol, and centrifuge. Pass through a nylon filter of 0.2-µm pore size, and discard the initial 1 mL of the filtrate.

**Sample solution**: Transfer 2.0 g of Ganoderma Lucidum Fruiting Body Powdered Extract RS in 5 mL of alcohol, and centrifuge. Pass through a nylon filter of 0.2-µm pore size, and discard the initial 1 mL of the filtrate. Evaporate to dryness under reduced pressure, and dissolve the residue in about 20 mL of alcohol. Transfer the solution to a 25-mL volumetric flask, dilute with alcohol to volume, and mix well. Pass through a nylon filter of 0.2-µm pore size, and discard the initial 1 mL of the filtrate. [NOTE—To facilitate the chromatographic column longevity, the following solid phase extraction procedure may be employed. Condition the solid phase extraction column containing about 200 mg of L1 packing with 5 mL of methanol followed by 3 mL of water; do not allow the column to dry. Transfer 2.0 mL of Ganoderma Lucidum Fruiting Body solution in alcohol into a 20-mL volumetric flask, dilute with water to volume, and mix well. Apply the entire volume onto the column, and elute at the rate of approximately 1 drop/s, employing vacuum. Rinse the column with 3 mL of water, and discard the rinsate. Elute with 2.0 mL of methanol and collect the eluate into the 2.0-mL volumetric flask. Adjust with methanol to volume, and mix well.] [NOTE—This method may result in coelution of ganoderic acid A and ganoderic acid K.]

**Chromatographic system**
(See Chromatography (621), System Suitability.)

**Mode**: LC

**Detector**: UV 257 nm

**Column**: 2.1-mm × 15-cm; 1.8-µm packing L1

**Column temperature**: 25°

**Flow rate**: 0.4 mL/min

**Injection volume**: 5 µL

**System suitability**

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

**Suitability requirements**

**Chromatographic similarity**: The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP Ganoderma Lucidum Fruiting Body Powdered Extract RS being used.

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

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

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

**Analysis**

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

[NOTE—Standard solution A, Standard solution B, and the Sample solution are 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 Powdered Extract RS being used, identify all specified ganoderic and ganoderenic acids in the Sample solution chromatogram. The approximate relative retention times, with respect to ganoderic acid A, are provided in Table 2.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganoderic 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>Ganoderic 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>Ganoderic 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 percentages of each triterpenoic acid in the portion of Ganoderma Lucidum Fruiting Body taken:

\[ \text{Result} = \left( \frac{r_F}{r_S} \right) \times C_s \times \left( \frac{V}{W} \right) \times F \times 100 \]

\[ r_F = \text{peak area of the relevant analyte from the Sample solution} \]

\[ r_S = \text{peak area of ganoderic acid A in Standard solution A} \]

\[ C_s = \text{concentration of USP Ganoderic Acid A RS in Standard solution A (mg/mL)} \]

\[ V = \text{volume of the Sample solution (mL)} \]

\[ W = \text{weight of Ganoderma Lucidum Fruiting Body taken to prepare the Sample solution (mg)} \]

\[ F = \text{relative response factor, with respect to ganoderic acid A (see Table 2)} \]

Calculate the sum of the percentages of all specified triterpenoic acids.

**Acceptance criteria**

**Sum of triterpenoic acids**: NLT 0.3% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures (233)**
  - **Acceptance criteria**
    - Arsenic: NMT 2.0 µg/g
    - Cadmium: NMT 1.0 µg/g
    - Lead: NMT 5.0 µg/g
    - Mercury: NMT 1.0 µg/g

- **Articles of Botanical Origin, General Method for Pesticide Residues Analysis (561)**: Meets the requirements

- **Microbial Enumeration Tests** (2021): The total aerobic bacterial count does not exceed 10^2 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^1 cfu/g.

- **Absence of Specified Microorganisms (2022)**: Meets the requirements of the tests for 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.
Sample solution: 0.5 mg/mL of 1-phenyl-3-methyl-5-pyrazolone in methanol

Internal standard solution: 0.5 mg/mL of D-lyxose in water

Standard stock solution: Composite solution containing 0.20 mg/mL each of USP Mannose RS, USP D-Glucuronic Acid RS, and USP Galactose RS; 2.0 mg/mL of USP Dextrose RS; and 0.10 mg/mL of USP L-Fucose RS in water

Standard solution: Combine 0.125 mL of Standard stock solution with 0.125 mL of Internal standard solution, 0.300 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°C for 30 min, and cool to room temperature. Add to the vial 0.300 mL of 0.15 M hydrochloric acid and 0.65 mL of water, mix well, and pass through a nylon filter of 0.45-µm or finer pore size.

NOTE—The amounts of individual analytes (Aᵢ) in the 0.125-mL aliquot of the Standard solution subjected to derivatization are approximately 0.25 mg for dextrose and 0.025 mg for mannose, galactose, and D-glucuronic acid.

Sample solution: Transfer 2.0 g of Ganoderma Lucidum Fruiting Body, finely powdered and accurately weighed, into a 200-mL round-bottom flask, add 60 mL of water, and allow to stand for 1 h. Attach a condenser, heat under reflux for 4 h, and filter immediately. Transfer the residue and the filter to the same 200-mL round-bottom flask. Add 60 mL of water, heat under reflux for 3 h, and filter immediately. Rinse the flask with three 5-mL portions of water, and filter. Combine the filtrates and the rinsates in a 250-mL beaker, and evaporate on the water bath to dryness. Dissolve the residue in 5 mL of water, add 75 mL of alcohol, mix well, allow to stand at 4°C for 12 h, and centrifuge at 4000 rpm for 30 min. Discard the supernatant, and dry the precipitate on a water bath. Dissolve the residue in hot water and quantitatively transfer into a 10-mL volumetric flask. Cool to room temperature, dilute with water to volume, and mix well. Centrifuge at 4000 rpm for 10 min. Accurately transfer 0.250 mL of the supernatant into a reaction vial, and add about 0.25 mL of 4 M trifluoroacetic acid. Seal the vial, heat at 110°C for 4 h, cool to room temperature, add 0.5 mL of methanol, and evaporate to dryness at 60°C under vacuum. Repeat the addition of 0.5 mL of methanol and subsequent evaporation three times. Add to the residue 0.125 mL of water, 0.125 mL of the Internal standard solution, 0.300 mL of 0.15 M sodium hydroxide solution, and 0.50 mL of the Reagent. Seal the vial, heat at 70°C for 30 min, and cool to room temperature. Add to the vial 0.300 mL of 0.15 M hydrochloric acid and 0.65 mL of water, mix well, and pass through a nylon filter of 0.45-µm or finer pore size.

Chromatographic system
(See Chromatography (621), System Suitability.)

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<thead>
<tr>
<th>Time (min)</th>
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<tbody>
<tr>
<td>0</td>
<td>84.0</td>
<td>16.0</td>
</tr>
<tr>
<td>30</td>
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</tr>
<tr>
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<td>16.0</td>
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</tbody>
</table>

Reagent: 0.1 M solution of 1-phenyl-3-methyl-5-pyrazolone in methanol

Internal standard solution: 0.5 mg/mL of D-lyxose in water

System suitability
Sample: Standard solution
Suitability requirements
Resolution: NLT 3.0 between the D-lyxose peak and the closest subsequent peak, and NLT 1.5 between the glucuronic acid peak and the closest preceding peak
Tailing factor: NMT 2.0 for the dextrose peak
Relative standard deviation: NMT 2.0% determined for the dextrose peak in replicate injections

Analysis
Samples: Standard solution and Sample solution

<table>
<thead>
<tr>
<th>Mode</th>
<th>Detector</th>
<th>Column</th>
<th>Flow rate</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>UV 250 nm</td>
<td>4.6-mm × 25-cm; 5-µm packing L1</td>
<td>1.0 mL/min</td>
<td>10 µL</td>
</tr>
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Chromatography

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<tr>
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<td>84.0</td>
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</tbody>
</table>

Change to read:

• BOTANICAL CHARACTERISTICS
  Macromorphological: Basidiocarp (fruiting body) morphology is highly variable. Shape of pileus (cap) ranges from reniform to subcircular, convex or concave, 15 cm or more broad, single to multiple layers thick (up to 3 cm); margin generally thick and blunt, sometimes acute. Pileus surface radially rugose (wrinkled) and concentrically sulcate.
  Stipe (stem) attachment predominately lateral; stipe length varies from very short to 10–12 cm long, 1–3 cm thick, cylindrical, reddish to almost black, laccate (lacquered). Hymenophore (pore surface) yellowish-white to tan/y. Pores small, circular to irregular, 4–7 per mm, 6–200 µm in diameter, distance between axes of pores about 260 µm.
**Microscopic:** Hyphal system trimitic with hyaline, thin-walled, clamped, septate generative hyphae, 1–4 µm in diameter, septa restricted to clamps, scantily branched, abundant at the growth margin of pileus and dissepiments (partitions). Skeletal hyphae are arboriform, aseptate, clampless, very long, 3–6 µm in diameter, scantily branched, branches with limited growth at distal end, with thick walls; they compose most of the context (flesh) and dissepiments, originating immediately behind the growth margin from generative hyphae. Binding hyphae of the “Bovista” type are aseptate, clampless, profusely branched, generally thinner and lighter than the skeletal, 1–3 µm in diameter. Basidiospores ovoid, double-walled, truncated at apex. Epispore thin, ovoid, hyaline, 9.0–11.5 × 6.0–8.0 µm; endospore thick, ovoid, 6.5–8.5 × 5.0–6.5 µm, bearing relatively few long and thick echinules that support the epispore, sometimes fused into a short crest.

- **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561):** NMT 2.0%
- **Loss on Drying (731)**
  Sample: 1.0 g of powdered Ganoderma Lucidum Fruiting Body
  Analysis: Dry at 105°C for 4 h.
  Acceptance criteria: NMT 17.0%
- **ARTICLES OF BOTANICAL ORIGIN, Total Ash (561)**
  Sample: 1.0 g of powdered Ganoderma Lucidum Fruiting Body
  Acceptance criteria: NMT 4.0%
- **ARTICLES OF BOTANICAL ORIGIN, Alcohol-Soluble Extractives, Method 1 (561)**

**Sample:** 2–4 g of powdered Ganoderma Lucidum Fruiting Body
**Acceptance criteria:** NLT 2.0%

- **ARTICLES OF BOTANICAL ORIGIN, Water-Soluble Extractives, Method 1 (561)**
  Sample: 2–4 g of powdered Ganoderma Lucidum Fruiting Body
  **Acceptance criteria:** NLT 3.0%

**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 binominal and, following the official name, the part of the fungus from which the article was derived.
- **USP REFERENCE STANDARDS (11)**
  USP Dextrose RS
  USP Ergosterol RS
  USP L-Fucose RS
  USP Galactose RS
  USP Ganoderic Acid A RS
  USP Ganoderma Lucidum Fruiting Body Powdered Extract RS
  USP D-Glucuronic Acid RS
  USP Mannose RS

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