Add the following:

**Ganoderma Lucidum Fruiting Body Powder**

**DEFINITION**

Ganoderma Lucidum Fruiting Body Powder is dried Ganoderma Lucidum Fruiting Body reduced to a powder or a very fine powder. It contains NLT 0.3% of triterpenoic acids, calculated on the dried basis as a sum of ganoderic acids A, B, C₂, D, F, G, and H and ganoderenic acids B, C, and D.

**IDENTIFICATION**

Change to read:

- **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 Powdered Extract RS in alcohol.
  
  Sonicate for about 10 min, centrifuge, and use the supernatant.
  
  **Sample solution:** Sonicate about 1 g of Ganoderma Lucidum Fruiting Body Powder in 50 mL of alcohol for 15 min, centrifuge, withdraw the supernatant, and evaporate to dryness under reduced pressure at 50°.

Dissolve the residue in 2.0 mL of alcohol, centrifuge, and use the supernatant.

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Mode:** HPTLC

**Adsorbent:** Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate).¹ 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 the Sample solution as 8-mm bands

**Temperature:** Ambient, not to exceed 30°

**Developing solvent system:** A mixture of toluene, ethyl formate, and formic acid (5: 5: 0.2)

**Spray reagent:** A solution of 10% sulfuric acid in alcohol. [NOTE—Prepare fresh. Slowly and gradually add sulfuric acid to ice-cold alcohol, and mix well.]

**System suitability**

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

**Suitability requirements**

**Chromatographic pattern:** Under long-wave UV (365 nm) and under white light, the chromatograms exhibit the band patterns approximately corresponding in color and position to those specified in Table 1.

**Retardation factor (Rᶠ) reproducibility:** The retardation factors obtained with **Standard solution A** and **Standard solution B** are within ±10% of the values specified in Table 1.

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1 Suitable commercially available plates are HPTLC Silica Gel 60 F₂₅₄ from EMD Millipore (e.g., Part No. 1.05642.0001).

---

**Table 1**

<table>
<thead>
<tr>
<th>Component</th>
<th>Retardation Factor (Rᶠ)</th>
<th>Long-wave UV Light (365 nm)</th>
<th>White Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>0.80</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>0.67</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.50</td>
<td>—</td>
<td>Orange</td>
</tr>
<tr>
<td>Ganoderic acid F</td>
<td>0.37</td>
<td>—</td>
<td>Bluish-green</td>
</tr>
<tr>
<td>Ganoderic acid D</td>
<td>0.31</td>
<td>—</td>
<td>Bluish-green</td>
</tr>
<tr>
<td>Ganoderic acid G</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ganoderic acid B</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ganoderic acid H</td>
<td>0.22</td>
<td>Yellow</td>
<td>—</td>
</tr>
<tr>
<td>Ganoderic acid A</td>
<td>0.16</td>
<td>Green to yellowish-green</td>
<td>—</td>
</tr>
<tr>
<td>Ganoderic acid C₂</td>
<td>0.13</td>
<td>—</td>
<td>Orange</td>
</tr>
<tr>
<td>Unknown</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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Analysis

Samples: Standard solution A, Standard solution B, and Sample solution. Apply the samples as bands, and dry in air. Develop in a saturated chamber until the solvent front has moved about four-fifths of the length of the plate, remove the plate from the chamber, air-dry, treat with Spray reagent, heat for about 5 min at 105°–110°C, and immediately examine under white light and under the long-wave UV light (365 nm).

Acceptance criteria: Under the long-wave UV light (365 nm) and under white light, the chromatogram of the Sample solution exhibits the bands corresponding in color and Retention time (RT) to similar bands in the chromatogram of Standard solution C. At the RT 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 Water-Soluble Polysaccharides.

Acceptance criteria: The chromatogram of the Sample solution exhibits peaks at the retention times corresponding to those of ganoderic acid C, ganoderic acid D, ganoderic acid G, ganoderic acid B, ganoderic acid A, ganoderic acid H, ganoderic acid D, ganoderic acid D, and ganoderic 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

Content of Tripterpenic Acids

Solution A: 0.075% Phosphoric acid in water
Solution B: Acetonitrile
Mobile phase: See Table 2.

<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>

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 RS 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 Powder, accurately weighed, to a 200-mL round-bottom flask, and add 75 mL of alcohol. Attach a condenser, reflux for 45 min, cool, and filter. Rinse the flask with two 10-mL portions of alcohol, and filter, combining the washes and 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 SPE column containing about 200 mg of L1 pack phase with 3 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 Powder solution in alcohol to 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, collect the eluate into the 2.0-mL volumetric flask, adjust with methanol to volume, and mix well.

NOTE—This method may result in co-elution 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 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 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 3.

Table 3

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganoderenic acid C</td>
<td>0.36</td>
<td>0.51</td>
</tr>
<tr>
<td>Ganoderic acid C</td>
<td>0.42</td>
<td>1.05</td>
</tr>
</tbody>
</table>
### CONTENT OF WATER-SOLUBLE POLYSACCHARIDES

#### SPECIFIC TESTS

#### Chromatographic system

- **Mode:** LC
- **Detector:** UV 250 nm
- **Column:** 4.6-mm × 25-cm; 5-μm packing L1
- **Column temperature:** 35°C
- **Flow rate:** 1.0 mL/min
- **Injection volume:** 10 μL

#### System suitability

- **Sample:** Standard solution
- **Suitability requirements:** NLT 1.5 between d-lyxose and closest subsequent peak, Standard solution; NLT 1.5 between glucuronic acid and closest preceding peak, Standard solution

#### Mobile phase:

- **Solution A:** Acetonitrile
- **Solution B:** 0.05 M phosphate buffer, pH 6.0

#### Table 4

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.0</td>
<td>16.0</td>
</tr>
<tr>
<td>30</td>
<td>82.5</td>
<td>17.5</td>
</tr>
<tr>
<td>55</td>
<td>81.0</td>
<td>19.0</td>
</tr>
<tr>
<td>60</td>
<td>81.0</td>
<td>19.0</td>
</tr>
<tr>
<td>61</td>
<td>84.0</td>
<td>16.0</td>
</tr>
</tbody>
</table>

### MICROBIAL ENUMERATION TESTS

- **Contaminants**
  - **Ninhydrin-reactive agents:** NMT 1.0 µg/mL
  - **Escherichia coli** tolerated Gram-negative bacteria count does not exceed 10⁵ cfu/g.

### ELEMENTAL IMPURITIES—PROCEDURES

- **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

- **Meets the requirements**

### MICROBIAL ENUMERATION TESTS

- **The total aerobic bacterial count does not exceed 10³ cfu/g,** and the bile-tolerant Gram-negative bacteria count does not exceed 10³ cfu/g.

### ABSENCE OF SPECIFIED MICROORGANISMS

- **Meets the requirements**

### SPECIFIC TESTS

#### CONTENT OF WATER-SOLUBLE POLYSACCHARIDES

- **Solution A:** 0.05 M phosphate buffer, pH 6.0
- **Solution B:** Acetonitrile
- **Mobile phase:** See Table 4.

#### Table 3 (Continued)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
</tr>
</thead>
<tbody>
<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 B</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 Powder taken:

\[
\text{Result} = \left( \frac{r_0}{r_s} \right) \times C_t \times \left( \frac{V}{W} \right) \times F \times 100
\]

- \( r_0 \) = peak area of the relevant analyte in the Sample solution
- \( r_s \) = peak area of ganoderic acid in Standard solution
- \( C_t \) = concentration of USP Ganoderic Acid A RS in Standard solution (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of Ganoderma Lucidum Fruiting Body Powder taken to prepare the Sample solution (mg)
- \( F \) = relative response factor, with respect to ganoderic acid A (see Table 3)

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 10³ cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10³ cfu/g.

- **Absence of Specified Microorganisms (2022):** Meets the requirements of the tests for absence of Salmonella species and Escherichia coli

### Chromatographic system

- **Mode:** LC
- **Detector:** UV 250 nm
- **Column:** 4.6-mm × 25-cm; 5-μm packing L1
- **Column temperature:** 35°C
- **Flow rate:** 1.0 mL/min
- **Injection volume:** 10 μL

#### System suitability

- **Sample:** Standard solution
- **Suitability requirements:** NLT 1.5 between d-lyxose and closest subsequent peak, Standard solution; NLT 1.5 between glucuronic acid and closest preceding peak, Standard solution

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

**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 the Standard stock solution with 0.125 mL of the 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 (A) in the 0.125 mL aliquot of the Standard solution submitted 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 Powder, accurately weighed, to 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, and pass through a nylon filter of 0.45-μm or finer pore size.
Tailing factor: NMT 2.0 for the dextrose peak, Standard solution
Relative standard deviation: NMT 2.0% determined for the dextrose peak in replicate injections, Standard solution

Analysis
Samples: Standard solution and Sample solution
[NOTE—The Standard solution and the Sample solution are stable for 24 h at room temperature.]

Using the chromatograms of the Standard solution and the reference chromatogram provided with the lot of USP Ganoderma Lucidum Fruiting Body Powdered Extract RS being used, identify the individual derivatized monosaccharides at about the following relative retention times, with respect to dextrose: 0.48 for mannose, 0.58 for xylose, 0.82 for d-glucuronic acid, 1.09 for galactose, and 1.35 for L-fucose.

Separately calculate the percentages of derivatized monosaccharides in the portion of Ganoderma Lucidum Fruiting Body Powder taken:

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

- \( R_i \): peak response ratio of the relevant analyte to the internal standard in the Sample solution
- \( R_s \): peak response ratio of the relevant analyte to the internal standard in the Standard solution
- \( A_i \): amount of the relevant analyte in the aliquot of the Standard solution subjected to derivatization (mg)
- \( F \): dilution factor to account for the sample aliquot submitted to derivatization (0.230 mL) relative to the volume of the Sample solution (10.0 mL), 40
- \( W \): weight of Ganoderma Lucidum Fruiting Body Powder taken to prepare the Sample solution (mg)

Calculate the sum of the percentages of mannose, d-glucuronic acid, dextrose, galactose, and L-fucose.

Acceptance criteria
Sum of monosaccharides: NLT 0.7% on the dried basis

- Botanical Characteristics: When milled, the fruiting body typically grinds into a fibrous mass, or fractures into tiny strips rather than a fine powder. Hypothial 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, clamped, 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, clamped, profusely branched, generally thinner and lighter than the skeletal, 1–3 μm in diameter. Basidiospores ovoid, double-walled, truncate 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 Powder
  - Analysis: Dry at 105° 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 Powder
  - 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 Powder
  - 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 Powder
  - 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 binomial 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 Mannose RS
  - USP D-Glucuronic Acid RS
  - USP Ganoderma Lucidum Fruiting Body Powdered Extract RS

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