Ganoderma lucidum Fruiting Body

Proposed For Comment Version 0.2

DEFINITION
The article consists of the dried fruiting bodies of *Ganoderma lucidum* (Curtis: Fr.) P. Karst. (Family Ganodermataceae). It contains NLT 0.3% of triterpenoic acid, calculated on the dried basis 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.

SYNONYMS
*Ganoderma lucidum* (Leyss.: Fr.) Karst.
*Polyporus lucidum* (Curtis: Fr.) Fr.

POTENTIAL CONFOUNDING MATERIALS
*Amauroderma rude*
*Amauroderma rugosum*
*Coriolus versicolor*
*Phellinus baumii*
Related *Ganoderma* species including *G. sinense* (*G. Japonicum*), *G. atrum*, *G. tsugae*, *G. applanatum*, *G. unguulatum*, *G. capense*, and *G. resinaceum*

SELECTED COMMON NAMES
Chinese: 灵芝，靈芝
English: Ganoderma, reishi, polypore luisant, ganoderme luisant
French: Polypore luisant, ganoderme luisant
German: Lackporling, glänzender lackporling
Japanese: 灵芝，霊芝，レイシ
Korean: 영지
Pinyin: Ling zhi, ling zhi cao
Spanish: Ganoderma, hongo pipa, hongo michoacano (Mexico)

CONSTITUENTS OF INTEREST
*Triterpenoic 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. BOTANIC CHARACTERISTICS

Macroscopic: Fruiting body, shape is highly variable, especially in cultivated samples. Specimens from North America are typically large and shelf-like while those from the tropics and Asia are usually smaller and consist of pileus (cap) and stipe (stalk). Pileus varies in shape from reniform to semi-rounded or subrounded and also from convex, to flat, to concave; 10–22 cm broad, 1–2 cm thick; upper surface smooth or with concentric ripples, corky, tough, with or without varnished appearance. Color varies, dark red to reddish-brown or reddish-black in center, yellowish, or light yellowish-brown toward the margin; edge is white when actively growing, thin, and sometimes slightly incurved; the flesh varies in color from yellowish-brown to dark brown. Pores are minute, dense (4–7 per mm), and whitish to brownish in color, 1–2 layers of spore-producing tubes, the length of tube layer varies from short (less than one third of the pileus thickness) to long (more than one third of the pileus thickness), each tube is 2–20 mm long. Spores are small and fine, yellowish-brown. When the stipe is present, the length varies, usually 4–10 cm long, 1–2 cm in diameter, often may be enlarged at base; same color and appearance as pileus surface; attachment of the stipe to the pileus varies from lateral to nearly central.

Microscopic:

Cross section: The outer part of the cap shows enlarged yellow-color hyphae. The underlying plectenchyma consists of very thin and densely packed hyphae. The inner part of the cap shows a network of gray-color hyphae approximately 2–7 \( \mu \text{m} \) in diameter. While most hyphae run horizontally, a few run vertically. The vertical spore-producing tubes at the lower side of the cap are dark brown in color, approximately 200 \( \mu \text{m} \) wide. Their inner surface is covered with the light hymenial line, forming dark brown basidiospores. The basidia are ellipsoidal or sphathulate, infrequent, thin-walled, and numerous. The spores are ovoid or elliptical with a dotted surface and up to 8 \( \mu \text{m} \) in length. The spore walls are doubled with the darker inner layer occasionally protruding through the outer hyaline layer, giving a shiny appearance.

• B. THIN-LAYER CHROMATOGRAPHY

Standard solution A: 1.0 mg/mL of USP Ganoderic Acid A RS in methanol
Standard solution B: 0.3 mg/mL of USP Ergosterol RS in methanol
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 1.0 g of *Ganoderma lucidum* Fruiting Body, finely powdered, 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\(^\circ\). 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 \( \mu \text{m} \) (HPTLC plates). Pre-develop the plate in methanol and dry at 105\(^\circ\) for 30 min.
- Application volume: 2 \( \mu \text{L} \) each of Standard solution A and Standard solution B, and 4 \( \mu \text{L} \) 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\(^\circ\)
- 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:\ an orange band; another orange band due to ganoderic acid C; a green to yellowish-green band due to ganoderic acid A and ganoderenic acid A at an R\ 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\ 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 R\ to the ganoderic acid A band in the chromatogram of Standard solution A; two reddish-violet bands, one at an R\ below that of ganoderic acid A (due to ganoderic acid C) 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\ 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\ 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\: two orange bands; a green to yellowish-green band at an R\ 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. 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\ corresponding to the band due to ergosterol in the chromatogram of Standard solution B.

Under visible light, Sample solution exhibits a bluish-violet band corresponding in R\ 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\ corresponding to that of the ergosterol band in the chromatogram of Standard solution B and one above the ergosterol band.

• C. UHPLC

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

Acceptance criteria: The Sample solution chromatogram exhibits peaks at the retention times corresponding to ganoderenic acid C, ganoderic acid C, 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. HPLC

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 Triterpenoic 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 minutes 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 Powdered 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:** Transfer about 2.0 g of *Ganoderma lucidum* Fruiting Body, finely powdered (capable of passing a 4-mm sieve) and accurately weighed, 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. Add 2 mL of this 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**
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±1º
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 Powdered Extract RS being used.
Resolution: NLT 1.0 between the ganoderic acid A and ganoderic 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 Powdered 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. Separately calculate the percentage of each of the triterpenoic acids in the portion of Ganoderma lucidum Fruiting Body taken. The approximate relative retention times, relative to ganoderic acid A, are provided in Table 2.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Retention Times</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>
</tbody>
</table>
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 solution (mL)
- \( W \): weight of Ganoderma lucidum Fruiting Body 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 triterpenoic acids as the sum of the percentages of the different triterpenoic acids.

**Acceptance criteria:** 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 <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>Ganoderic acid D</th>
<th>1.33</th>
<th>1.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganoderic acid F</td>
<td>1.54</td>
<td>1.45</td>
</tr>
<tr>
<td>Time (min)</td>
<td>Solution A (%)</td>
<td>Solution B (%)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<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 stock solution A: 0.2 mg/mL of USP Mannose RS in water

Standard solution A: Transfer 0.125 mL of Standard stock solution A to a reaction vial and proceed as directed for the Sample solution beginning with — add 0.125 mL of Internal standard solution.

Standard stock solution B: 0.2 mg/mL of USP D-Glucuronic Acid RS in water

Standard solution B: Transfer 0.125 mL of Standard stock solution B to a reaction vial and proceed as directed for the Sample solution beginning with — add 0.125 mL of Internal standard solution.

Standard stock solution C: 2.0 mg/mL of USP Dextrose RS in water

Standard solution C: Transfer 0.125 mL of Standard stock solution C to a reaction vial and proceed as directed for the Sample solution beginning with — add 0.125 mL of Internal standard solution.

Standard stock solution D: 0.2 mg/mL of USP Galactose RS in water

Standard solution D: Transfer 0.125 mL of Standard stock solution D to a reaction vial and proceed as directed for the Sample solution beginning with — add 0.125 mL of Internal standard solution.

Standard stock solution E: 0.1 mg/mL USP L-Fucose RS in water

Standard solution E: Transfer 0.125 mL of Standard stock solution E to a reaction vial and proceed as directed for the Sample solution beginning with — add 0.125 mL of Internal standard solution.

Sample solution: Transfer 2.0 g of Ganoderma lucidum Fruiting Body, finely powdered (capable of passing a 4-mm sieve), and accurately weighed, 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 with three 5-mL portions 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 4º 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 110º for 4 h, cool to room temperature, add 0.50 mL of methanol, and evaporate to dryness at 60º under vacuum. Repeat the addition of 0.50 mL of methanol and evaporation three times. Dissolve the residue in 0.125 mL of water. Add 0.125 mL of Internal standard solution, 0.3 mL of 0.15 M sodium hydroxide solution, and 0.5 mL of Reagent, seal the vial, and mix well on a vortex mixer. Heat at 70º for 30 min, cool to room temperature, and add 0.3 mL of 0.15 M hydrochloric acid and mix. Quantitatively transfer the solution to a 2-mL volumetric flask and dilute with water to volume. Before injection, pass through a membrane filter having a 0.45-μm or finer pore size and discard the first part of the filtrate.

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: 35º±1
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: Standard solution C
Suitability requirements
   Tailing factor: NMT 2.0 for the dextrose peak, Standard solution C
   Relative standard deviation: NMT 2.0%, determined from dextrose peak in repeated injections, Standard solution C

Analysis
Using the chromatograms of Standard solution A, Standard solution B, Standard solution C, Standard solution D, and Standard solution E, identify the retention times of the peaks corresponding to mannose, glucuronic acid, glucose, galactose, and fucose in the Sample solution chromatogram.
Separately calculate the percentages of mannose, glucuronic acid, glucose and galactose in the portion of Ganoderma lucidum Fruiting Body taken:

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

\[R_u\] = peak response ratio of the relevant analyte to the internal standard from the Sample solution
\[R_s\] = peak response ratio of the analyte to the internal standard from the relevant Standard solution
\[C_s\] = concentration of the analyte in the relevant Standard stock solution (mg/mL)
\[V\] = volume of the Sample solution (mL)
\[W\] = weight of Ganoderma lucidum Fruiting Body taken to prepare the Sample solution (mg)

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

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

Acceptance criteria: NLT 0.7% on the dried basis

• Articles of Botanical Origin, Foreign Organic Matter <561>: NMT 2.0%
• Loss on Drying <731>
   Analysis: Dry 1.0 g of Ganoderma lucidum Fruiting Body, finely powdered, at 105° for 4 h.
   Acceptance criteria: NMT 17.0%
• Articles of Botanical Origin, Alcohol-Soluble Extractives, Method 1 <561>: NMT 2.0%
• Articles of Botanical Origin, Water-Soluble Extractives, Method 1 <561>: NLT 3.0%
• Articles of Botanical Origin, Total Ash <561>
   Analysis: 3.0 g of Ganoderma lucidum Fruiting Body, finely powdered
   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 part of the plant contained in the article.

• **USP Reference Standards**<11>

  USP Dextrose RS [1]
  USP Ergosterol RS [2]
  USP L-Fucose RS [3]
  USP Galactose RS [4]
  USP Ganoderic Acid A RS
  USP D-Glucuronic Acid RS [5]
  USP Mannose RS [6]
  USP *Ganoderma lucidum* Fruiting Body Powdered Extract RS

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