Add the following:

■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_2, 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, finely powdered, 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 (R_F) reproducibility: The retardation factors obtained with *Standard solution A* and *Standard solution B* are within ±10% of the values specified in *Table 1*.

[1] Suitable commercially available plates are HPTLC Silica Gel 60 F_{254} from EMD Millipore (e.g., Part No. 1.05642.0001).

Table 1

Component	Retardation Factor (R_F)	Long-wave UV Light (365 nm)			White Light		
		Standard solution A	Standard solution B	Standard solution C	Standard solution A	Standard solution B	Standard solution C
Unknown	0.80	—	—	Bluish-green	—	—	—
Ergosterol	0.67	—	Blue	Blue	—	Blue	Blue
Unknown	0.50	—	—	Orange	—	—	Bluish-violet
Ganoderic acid F	0.37	—	—	—	—	—	—
Ganoderic acid D Ganoderenic acid D	0.31	—	—	Bluish-green	—	—	Bluish-green
Ganoderic acid G Ganoderic acid B Ganoderenic acid B Ganoderic acid H	0.22	—	—	Yellow	—	—	Reddish-violet
Ganoderic acid A Ganoderenic acid A	0.16	Green to yellowish-green	—	Green to yellowish-green	Bluish-violet	—	Bluish-violet
Ganoderic acid C_2 Ganoderenic acid C	0.13	—	—	Orange	—	—	Reddish-violet
Unknown	—	—	—	Orange	—	—	—

[NOTE—The Standard solutions are stable for 72 h at room temperature.]

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°, 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 R_F to similar bands in the chromatogram of *Standard solution C,* at the R_F 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.]

(Postponed indefinitely)● (RB 1-Aug-2014)

- **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 C_2, 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 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 TRITERPENOIC ACIDS**

Solution A: 0.075% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80.0	20.0
3	73.5	26.5
34	73.5	26.5
52	61.5	38.5
53	80.0	20.0
58	80.0	20.0

[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 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, finely powdered and accurately weighed, to a 200-mL round-bottom flask, 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 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 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 ganoderenic 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

Analyte	Relative Retention Time	Relative Response Factor
Ganoderenic acid C	0.36	0.51
Ganoderic acid C_2	0.42	1.05

Table 3 *(Continued)*

Analyte	Relative Retention Time	Relative Response Factor
Ganoderic acid G	0.56	1.18
Ganoderenic acid B	0.60	0.45
Ganoderic acid B	0.66	1.10
Ganoderic acid A	1.00	1.00
Ganoderic acid H	1.05	1.54
Ganoderenic acid D	1.25	0.51
Ganoderic acid D	1.33	1.08
Ganoderic acid F	1.54	1.45

Separately calculate the percentages of each triterpenoic acid in the portion of Ganoderma Lucidum Fruiting Body taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte in the *Sample solution*
r_S = peak area of ganoderic acid in *Standard solution A*
C_S = concentration of USP Ganoderic Acid A RS 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 = 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 10^5 cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed 10^3 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 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	84.0	16.0
30	82.5	17.5
55	81.0	19.0
60	81.0	19.0
61	84.0	16.0

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
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° 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_S) 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, finely powdered and 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, allow to stand at 4° 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, quantitatively transfer to 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 to a reaction vial, and add about 0.25 mL of 4 M trifluoroacetic acid. Seal the vial, heat at 110° for 4 h, cool to room temperature, add 0.5 mL of methanol, and evaporate to dryness at 60° 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 *Reagent*. Seal the vial, heat at 70° 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*.)
Mode: LC
Detector: UV 250 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: 1.0 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.5 between D-lyxose and closest subsequent peak, *Standard solution*; NLT 1.5 between glucuronic acid and closest preceding peak, *Standard solution*

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 lyxose, 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 taken:

$$\text{Result} = (R_U/R_S) \times A_S \times (F/W) \times 100$$

R_U = 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_S = 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 (0.250 mL) relative to the volume of the *Sample solution* (10.0 mL), 40

W = weight of Ganoderma Lucidum Fruiting Body 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**

Macroscopic: 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 culcate; shiny, yellowish-red to reddish-black. Stipe (stem) attachment predominantly 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 tawny. 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, 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

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

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 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 Ganoderic Acid A RS
USP Ganoderma Lucidum Fruiting Body Powdered Extract RS
USP D-Glucuronic Acid RS
USP Mannose RS

■2S (*USP37*)