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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, C₂, D, F, G, and H and ganoderenic acids B, C, and D.

IDENTIFICATION

• 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 Predevelop 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

Column temperature: Ambient, not to exceed 30°

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

Developing distance: 6 cm

Derivatization 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 light (365 nm), the chromatogram of *Standard solution C* displays, in the bottom third of the plate, the following bands in the order of increasing R_f : a yellowish or orange band (sometimes, two orange bands are seen); a bluish-green band corresponding to the light-blue ganoderic acid A band in *Standard solution A*; an intense yellow band corresponding to ganoderic acid B, ganoderic acid G, ganoderic acid H, and ganoderenic acid B; and a bluish-green band coincident with ganoderic acid D and ganoderenic acid D. In the middle third of the chromatogram, a variable number of blue-green bands appear. At the top of the middle third of the *Standard solution C* chromatogram, a somewhat diffuse band coincident with the ergosterol band in *Standard solution B* is seen. In the upper third of the chromatogram, three or four diffuse bands of varying colors appear. Under white light, *Standard solution C* exhibits, in its lower third, two brownish-red bands, the upper of them coincident with the ganoderic acid A band in *Standard solution A*, followed by a more intense brown band; and a lighter brown band corresponding to ganoderic acid D and ganoderenic acid D. In the middle third of the chromatogram, five or six light-brown bands are seen; one of those, deepest in color and relatively diffuse, corresponds to the ergosterol band in *Standard solution B*. 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 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.]

• **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, 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 1](#).

Table 1

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 rinsates 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 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 ganoderenic acid A and ganoderic acid K.]

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC