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Traditional Chinese medicine — *Ganoderma lucidum* fruiting body

Médecine traditionnelle chinoise — Organe fructifère de Ganoderma lucidum

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Ganoderma lucidum fruiting body is the dried fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst., which is also known as Lingzhi or Reishi. It has been used as a medicinal mushroom in many Asian countries for more than 2000 years, such as China, Japan and Korea, making it one of the oldest mushrooms known to have been used medicinally. This fungus can tonify Qi, and is traditionally used to treat fatigue, cough and insomnia in traditional Chinese medicine. Modern pharmacological studies also demonstrate its great potential in tumour treatment and immuno-enhancement. Therefore, the market of *Ganoderma lucidum* has developed very rapidly, as indicated by the increase in yield, production output and trade volume.

However, there remain many challenges, such as adulteration of similar species, and lack of suitable testing methods for quality assessment. In addition, though *Ganoderma lucidum* has been recorded in several pharmacopoeias, such as Chinese Pharmacopoeia, United States Pharmacopeia Herbal Medicines Compendium and the Korean Herbal Pharmacopoeia, the specifications and quality requirements in these standards vary. Therefore, there is a clear and urgent need to develop an international standard for harmonizing the existing standards, as well as ensuring the safety and effectiveness of *Ganoderma lucidum* fruiting body.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.2.3, 5.2.4 and 5.2.5 in their national standards. Examples of national and regional values are given in Annex E.

Traditional Chinese medicine — *Ganoderma lucidum* fruiting body

1 Scope

This document specifies minimum requirements and test methods for *Ganoderma lucidum* fruiting body that is derived from *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

It is applicable to *Ganoderma lucidum* fruiting body that is sold and used as Chinese materia medica (whole medicinal materials) and decoction pieces derived from this fungus.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1575, *Tea — Determination of total ash*

ISO 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO 20409, *Traditional Chinese medicine — Panax notoginseng root and rhizome*

ISO 21371, *Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use*

CAC/MRL01, *Maximum Residue Limits for Pesticides in Food*

CODEX STAN 229, *Analysis of pesticide residues: Recommended methods*

World Health Organization. 2011, *Quality control methods for herbal materials, General advice on sampling*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

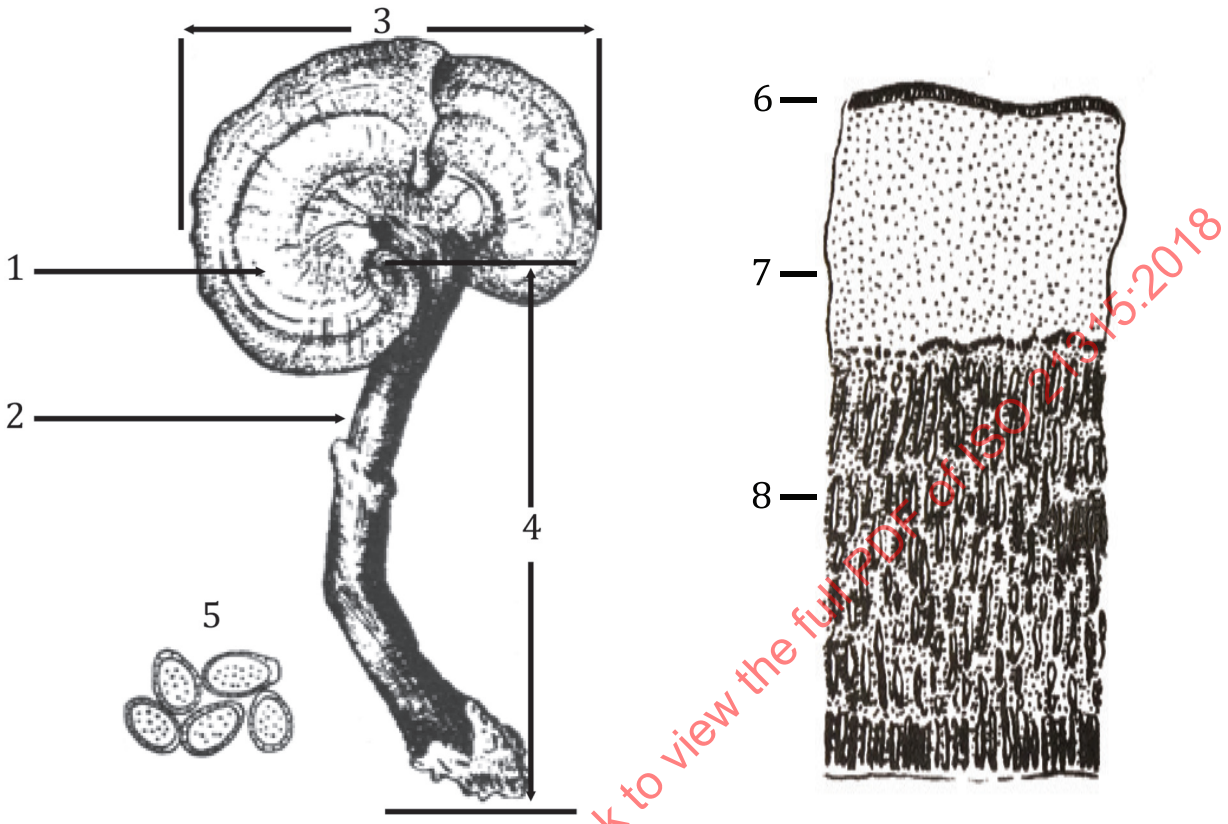
— IEC Electropedia: available at http://www.electropedia.org/

3.1
fruiting body
dried sporocarp of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

3.2
reference standard
authentic substance used as a measurement base for TLC identification or marker compound quantification

4 Descriptions

Ganoderma lucidum fruiting body is the dried fruiting body of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. in the family of Ganodermataceae shown in Figure 1.



a) Fruiting body of *Ganoderma lucidum* **b) Vertical section of pileus**

Key

1 pileus
2 stipe
3 pileus diameter
4 stipe length
5 spore
6 crust
7 context
8 tube

Figure 1 — Structure of *Ganoderma lucidum*

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling.

a) *Ganoderma lucidum* fruiting body shall be clean and free from foreign matter.

b) The presence of living insects, moulds and external contaminants which are visible to the naked eye shall not be permitted.

5.2 *Ganoderma lucidum* fruiting body

5.2.1 Morphological features of fruiting body

a) Outline of fruiting body umbrella-shaped, pileus reniform, semi-rounded or surrounded, 10 cm to 30 cm in diameter, 1 cm to 4 cm thick.

b) Colour of pileus varies, yellowish-brown to reddish-brown, lustrous, with circular stripe and radiate wrinkle.

c) Stipe cylinder, usually 4 cm to 15 cm long, 1 cm to 3,5 cm in diameter, reddish-brown, luminous; attachment of the stipe to the pileus varies from lateral to nearly central.

d) Spore small and fine, yellowish-brown.

e) Odour slightly aromatic, taste slightly bitter.

f) Crust hard, colour similar to pileus or darker, the context varies in colour from yellowish-brown to dark brown, 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).

5.2.2 Thin-layer chromatogram (TLC) identification

The identification of *Ganoderma lucidum* fruiting body with thin-layer chromatogram (TLC) shall present the spot or band with the same colour and position corresponding to those of reference standard solution.

5.2.3 Moisture

The mass fraction of moisture should not be more than 17,0 %.

5.2.4 Total ash

The mass fraction of total ash should not be more than 4,0 %.

5.2.5 Water-soluble extractives

The mass fraction of water-soluble extractives should not be less than 3,0 %.

5.2.6 Marker compounds

Marker compounds such as polysaccharides and ganoderic acid A shall be determined.

5.2.7 Heavy metals

The contents of heavy metals such as arsenic, cadmium, lead and mercury shall be determined.

5.2.8 Pesticide residues

The content of pesticide residues such as benzex, DDT (dichloro-diphenyl-trichloroethane) and quintozene shall be determined.

6 Sampling

Sampling of *Ganoderma lucidum* fruiting body shall be in accordance with the World Health Organization 2011 *Quality Control Methods for Herbal Materials, General Advice on Sampling.*

7 Test methods

7.1 Macroscopic identification

Samples not less than 500 g are taken from each batch randomly. These samples are examined by the naked eye, smell and taste.

7.2 TLC identification

See Annex A for additional information.

7.3 Determination of moisture content

The testing method specified in ISO 20409 shall apply.

7.4 Determination of total ash

The testing method specified in ISO 1575 shall apply.

7.5 Determination of water-soluble extractives

See Annex B for additional information.

7.6 Determination of marker compounds

See Annex C and D for additional information.

7.7 Determination of heavy metals

The testing method specified in ISO 18664 shall apply.

7.8 Determination of pesticide residues

The testing method specified in CAC/MRL01 and CODEX STAN 229 shall apply.

8 Test report

For each test method, the test report shall specify the following:

a) all information necessary for the complete identification of the sample;

b) the sampling method used;

c) the test method(s) used;

d) the test result(s) obtained;

e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

g) the date of the test.

9 Packaging, storage and transportation

The packaging shall not transmit any flavour or odour to the product and shall not contain substances which may damage the product or constitute a health risk.

The product shall be stored in a dry and cool place.

The product shall be protected from light, moisture, pollution and entry of foreign substances during long distance delivery.

10 Marking and labelling

The requirements specified in ISO 21371 shall apply. The following items shall be marked or labelled on the packages:

a) product name;

b) category of the product in the marketed country or region;

c) net weight/quantity;

d) contact information;

e) name of raw materials;

f) warning statements, if any;

g) expiry date;

h) storage method;

i) batch/lot number;

j) miscellaneous.

Annex A
(informative)

TLC identification

A.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Weigh approximately 2 g of the powder, add 50 ml of petroleum ether, ultrasonicate for 30 min, filter, discard the filtrate, and evaporate the residue to dryness. Sonicate the residue in 80 ml of trichloromethane for 30 min, centrifuge, and evaporate the supernatant to dryness. Dissolve the residue in 2,0 mL of methanol and take supernatant as the test solution.

A.2 Preparation of reference standard solution

Dissolve ganoderic acid A in methanol to prepare the reference standard solution containing 0,5 mg/ml ganoderic acid A.

A.3 Developing solvent system

Prepare a mixture of n-hexane, ethyl acetate, methanol, and formic acid in the volume ratio of 30:30:2:0,2 as the first mobile phase. Prepare a mixture of n-hexane, ethyl acetate, methanol, and formic acid in the volume ratio of 30:30:1:0,2 as the second mobile phase.

A.4 Identification by TLC

Use silica gel as the adsorbent for the thin-layer plate. Apply separately to the plate 3 µl of the sample solution and the reference standard solution. Develop the chromatograms using the first mobile phase with a developing distance of 5 cm. Remove the plate from the chamber, and dry. Then, develop the chromatograms again using the second mobile phase with a developing distance of 8 cm, remove the plate from the chamber, and dry. Treat with 10 % sulfuric acid-ethanol, and heat for about 3 min at 105 °C. Immediately examine under UV light at 365 nm. The chromatogram of the sample solution exhibits bands corresponding in colour and position to similar bands in the chromatogram of the reference standard solution. Typical TLC chromatograms are shown in Figure A.1. The reference standard solution exhibits a green to yellowish-green band due to ganoderic acid A.

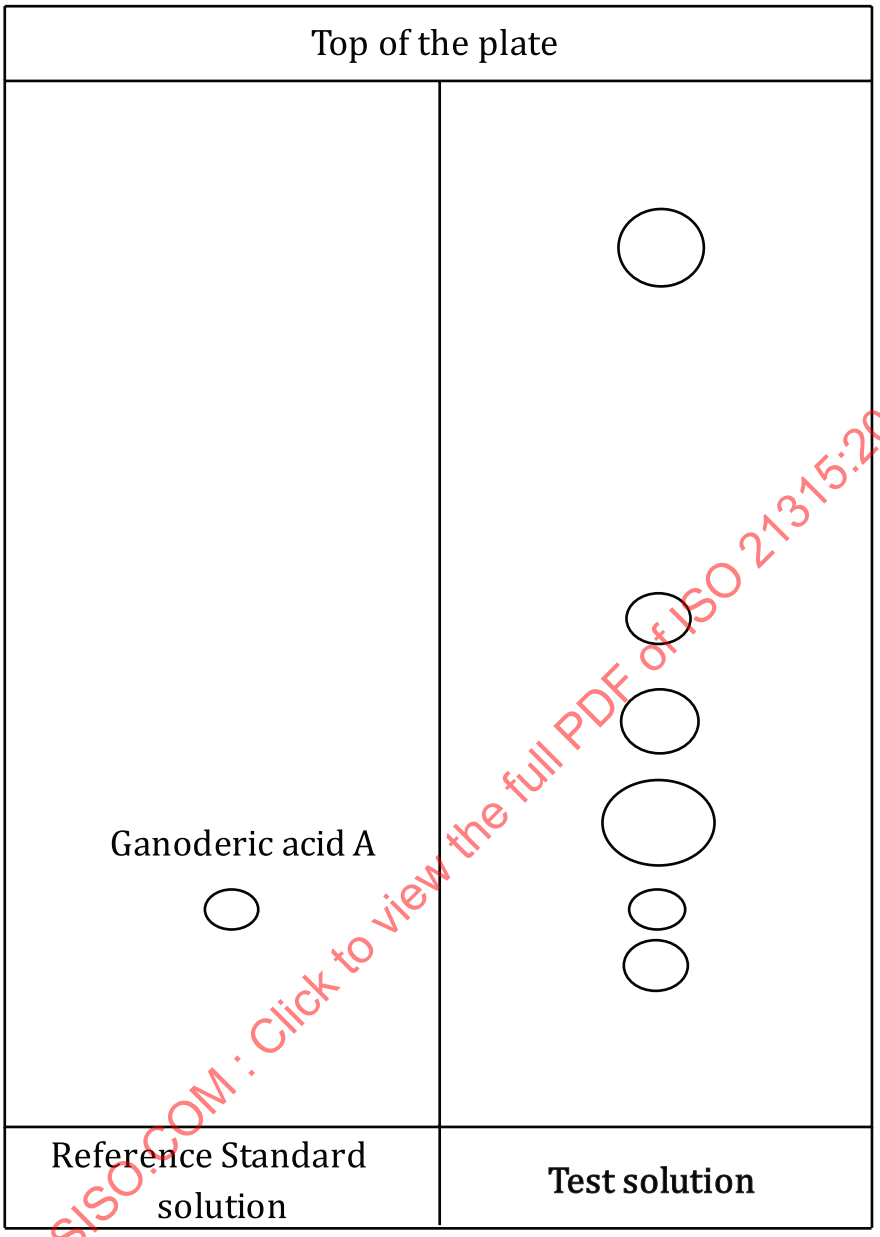


Figure A.1 — Schematic diagram of typical TLC chromatograms of *Ganoderma lucidum* fruiting body

Annex B
(informative)

Determination of water-soluble extractives

a) Weigh 250 g of the sample to grind and pass it through a 24 mesh or coarse sieve. Weigh approximately 4 g of the powder into a 250-ml stopper conical flask. Add accurately 50 ml water. Weigh and allow to stand for 1 h.

b) Heat it under reflux to slightly boil on a water bath for 1 h. Cool and weigh again. Replenish the loss of weight with water, mix well and filter.

c) Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into the evaporating dish. Evaporate the filtrate to dryness on a water bath.

d) Dry at 105 °C for 3 h and allow to cool for 30 min in a desiccator. Weigh the extracts rapidly and accurately.

e) Calculate the mass fraction of water-soluble extractives, w_{wse}, on the dried basis (%) with Formula (B.1).

$$w_{wse}(\%) = (m_1 - m_0) \times 2 / m_s \times 100 \quad \text{(B.1)}$$

where

m_1 is the mass of the evaporating dish and residue after drying (g);

m_0 is the mass of the evaporating dish (g);

m_s is the mass of the sample (g).

Annex C
(informative)

Determination of polysaccharides

C.1 Principle of the test method

The anthrone-sulfuric acid method is used to determine the content of polysaccharides. In this method, the concentrated sulfuric acid breaks down the polysaccharides to monosaccharides. Pentoses (5-carbon monosaccharides) are then dehydrated to furfural, and hexoses (6-carbon monosaccharides) to hydroxymethyl furfural. These compounds then react with anthrone to produce blue-green complexes that can be measured spectrophotometrically. Glucose is generally used as the reference standard in this method. Therefore, in this method, the content of the monosaccharide (glucose) can reflect the content of polysaccharides.

C.2 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Transfer 2,0 g of the powder, 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 three times with 5 ml 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 ethanol, mix well, allow to stand at 4 °C for 12 h, and centrifuge at 4 000 rpm for 30 min. Discard the supernatant and dry the precipitate. Dissolve the residue in hot water, quantitatively transfer the solution to a 50-ml volumetric flask, dilute with water to volume, and mix. Centrifuge the solution at 4 000 rpm for 10 min. Transfer 3,0 ml of the supernatant, accurately measured, to a 25-ml volumetric flask, dilute with water to volume, and mix.

C.3 Preparation of reference standard solution

Accurately weigh a quantity of anhydrous glucose to a measuring flask, dissolve in water to prepare a solution containing 0,12 mg anhydrous glucose per millilitre as the reference standard solution.

C.4 Construction of calibration curve

Separately transfer 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml, 1,0 ml and 1,2 ml of the reference standard solution to 10-ml test tubes with glass stopper, dilute with water to 2,0 ml, add 6,0 ml of freshly prepared anthrone-sulfuric acid solution (0,1 g of anthrone in 100 ml of sulfuric acid), and mix. Allow to stand for 15 min, and cool the tube in an ice bath for 15 min. Determine the absorbance of the samples at 625 nm using an ultraviolet-visible spectrophotometer. Construct the calibration curve by plotting the absorbance (Y-axis) against the concentration of the glucose (X-axis).

C.5 Content of polysaccharides

Transfer 2,0 ml of the sample solution to a 10-ml test tube with glass stopper, and determine the absorbance according to the method in C.4 (beginning from “add 6,0 ml of freshly prepared anthrone-

sulfuric acid solution"). Calculate the content of glucose in sample solutions using the calibration curve. The content of polysaccharides is calculated with Formula (C.1):

$$w_{\text{pol}}\,(\%) = \frac{\frac{(a-b)}{c} \times 50 \times 25}{m_{\text{s}} \times 3 \times 10^{3} \times (1 - w_{\text{M}})} \times 100 \qquad \text{(C.1)}$$

where

a is the absorbance of the test solution;

b is the intercept of the calibration curve;

c is the slope of the calibration curve;

m_{s} is the mass of the sample;

w_{M} is the moisture content of the sample (%);

w_{pol} is the content of polysaccharides (%).

Annex D
(informative)

Determination of ganoderic acid A

D.1 Preparation of test solution

Weigh 250 g of sample to grind, and pass it through an 80 mesh or finer sieve. Weigh accurately 2 g of the powder, add 50 ml of 95 % ethanol, heat and reflux for 1 h. Filter and evaporate the filtrate to dryness under reduced pressure at 60 °C. Dissolve the residue in 10 ml of methanol. Before injection, pass through a filter having a 0,45-µm pore size, and discard the first portion of the filtrate.

D.2 Preparation of reference standard solution

Accurately weigh a quantity of ganoderic acid A to a measuring flask, dissolve in a mixture of methanol to prepare a solution containing 0,1 mg per millilitre as the reference standards solution.

D.3 Apparatus and chromatographic conditions

D.3.1 Column, with a stationary phase of octadecylsilane bonded silica gel or equivalent.

Size: l = 250 mm, $Ø$ = 4,6 mm, particle size = 5 µm.

Theoretical plates: not less than 10 000.

Column temperature: 15 °C.

D.3.2 Detector: UV 254 nm.

D.3.3 Flow rate: 1 ml/ml.

D.3.4 Injection volume: 10 µL.

D.3.5 Mobile phase.

D.3.5.1 Mobile phase A: acetonitrile.

D.3.5.2 Mobile phase B: 0,05 mol/L ammonium dihydrogen phosphate in water.

D.3.5.3 Gradient program.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	**18,5**	81,5
8	**25,5**	74,5
90	**34,5**	65,5