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Traditional Chinese medicine — *Poria cocos* sclerotium

Médecine traditionnelle chinoise — Poria cocos *sclerotium*

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Contents

Page

Foreword

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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

Poria cocos sclerotium is the dried sclerotium of the fungus *Poria cocos* (Schw.) Wolf (Polyporaceae). It is a medicinal herb which has been widely used as functional food and crude drug to promote urination to drain dampness, fortify the spleen and calm the heart in Asian countries for thousands of years.

The products of *Poria cocos* sclerotium are sold all over the world. There are at least 23 countries and regions using *Poria cocos* sclerotium and its products. Major users include China, Japan, the Republic of Korea, Viet Nam, Malaysia and Singapore. Factors including producing areas, processing, packaging and storage conditions affect the quality of *Poria cocos* sclerotium. The quality of *Poria cocos* in the market can be unstable.

Poria cocos sclerotium is recorded in the Pharmacopoeia of the People's Republic of China[1], the European Pharmacopoeia[5], the Japanese Pharmacopoeia[2] and the Korean Pharmacopoeia[4]. However, the requirements and test methods of *Poria cocos* in these national and regional standards are varied and can cause barriers to international trade. In addition, due to its great demand in the global market, trade in *Poria cocos* sclerotium can be complicated by adulteration and substitution issues. The establishment of an International Standard for *Poria cocos* sclerotium is therefore necessary to ensure quality consistency, support clinical safety and effectiveness and promote international trade.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.6 and 5.7 in their national standards. Examples of national and regional values are given in Annex C.

Traditional Chinese medicine — *Poria cocos* sclerotium

1 Scope

This document specifies the quality, safety requirements and test methods for *Poria cocos* sclerotium that is derived from the fungus *Poria cocos* (Schw.) Wolf.

It is applicable to *Poria cocos* sclerotium sold and used as natural medicines in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces.

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

ISO/TS 21310, *Traditional Chinese medicine — Microscopic examination of medicinal herbs*

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

ISO 22217, *Traditional Chinese medicine —Storage requirements for raw materials and decoction pieces*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23723, *Traditional Chinese medicine — General requirements for herbal raw material and materia medica*

3 Terms and definitions

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

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

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

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

3.1
***Poria cocos* sclerotium**
dried sclerotium of the fungus *Poria cocos* (Schw.) Wolf (Polyporaceae) (syn. *Wolfiporia cocos* (F.A. Wolf) Ryvarden & Gilb.; *Wolfiporia extensa* (Peck) Ginns)

3.2
whole poria
whole dried *Poria cocos sclerotium* (3.1) with *skin* (3.5)

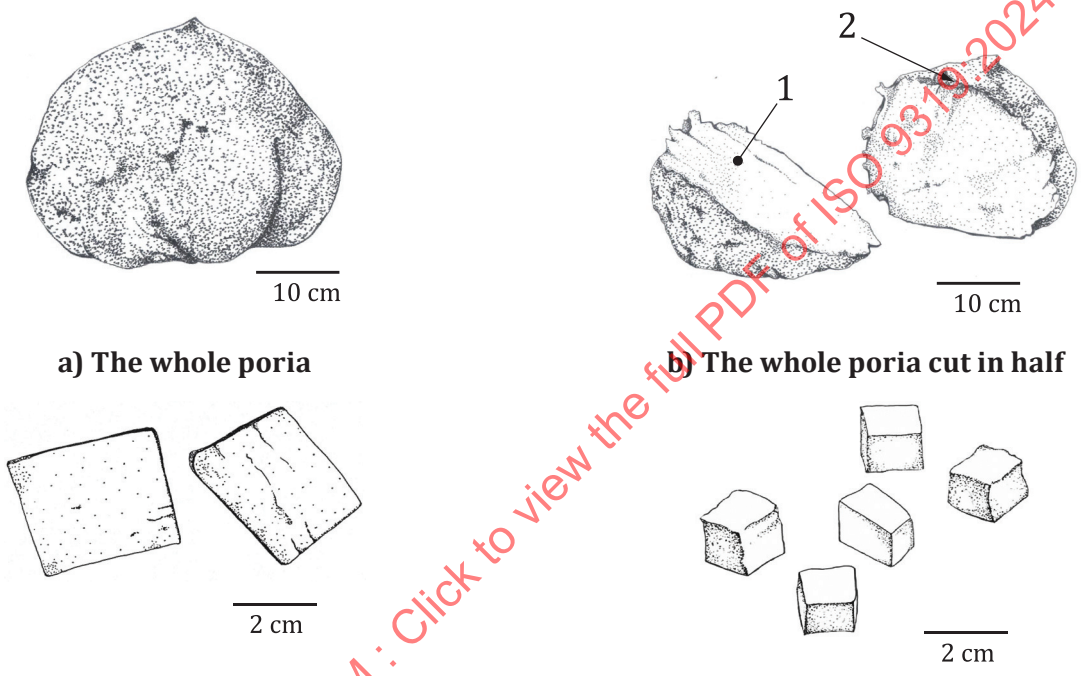
3.3
cubic poria
peeled *Poria cocos sclerotium* (3.1) without *skin* (3.5) cut in cubic pieces, variable in size

3.4
sliced poria
peeled *Poria cocos sclerotium* (3.1) without *skin* (3.5) cut in irregular thick slices, varying in thickness

3.5
skin
outer layer of the *whole poria* (3.2)

4 Descriptions

Poria cocos sclerotium is the dried sclerotium of the fungus *Poria cocos* (Schw.) Wolf (Polyporaceae) and has different trade forms, including whole poria, cubic poria, sliced poria and powdered poria, as shown in Figure 1.



a) The whole poria

b) The whole poria cut in half

c) Sliced poria

d) Cubic poria

Key
1 inner part
2 skin

Figure 1 — Structure of *Poria cocos* sclerotium

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Poria cocos* sclerotium shall be clean and free from foreign matter.

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

5.2 Morphological features

5.2.1 Whole poria

Whole poria is subglobose, ellipsoid, oblate or irregular-shaped and variable in size. The skin is thin and rough, brown to blackish-brown, conspicuously shrivelled and striated. The texture is hard and compact, the fracture granular, sometimes cracked, the outer layer pale brown, the inner part white or occasionally reddish, with some showing the penetrating roots of pine in the centre. The odour is slight, the taste weak and it is sticky when chewed.

5.2.2 Cubic poria

Cubic poria are cubic pieces or cubic thick slices, variable in size. The colour is white, pale red or pale brown. The odour is slight, the taste weak and they are sticky when chewed.

5.2.3 Sliced poria

Sliced poria are irregular thick slices, variable in thickness. The colour is white, pale red or pale brown. The odour is slight, the taste weak and they are sticky when chewed.

5.3 Microscopic identification

The powdered *Poria cocos* sclerotium is whitish with a pale brown hue. Examined under a microscope using chloral hydrate solution, the powder shows an irregularly shaped and occasionally granular and branched colourless mass, which dissolves gradually in chloral hydrate solution. The hyphae are colourless or pale brown, slender, slightly curved, branched and 3 µm to 8 µm (occasionally up to 16 µm) in diameter, as shown in Figure 2.

a) Granular and branched mass **b) Hyphae**

Figure 2 — Structure of granular and branched mass and hyphae

5.4 Thin-layer chromatography (TLC)

The main spots in the chromatogram obtained with the test solution correspond in position and colour to the spots obtained with the reference solutions.

5.5 Chemical colour reaction

When one drop of iodinated potassium iodide solution is added to a small piece or powder of sample, a deep red colour shall be produced.

5.6 Moisture

The moisture should not be more than a mass fraction of 15,0 %.

5.7 Total ash

The total ash should not be more than a mass fraction of 2,0 %.

5.8 Extractives

5.8.1 Ethanol-soluble extractives

The mass fraction of dilute ethanol-soluble extract should be determined.

5.8.2 Water-soluble extractives

The mass fraction of water-soluble extract should be determined.

5.9 Content of marker compound(s)

The content of marker compound(s) such as pachymic acid should be determined.

5.10 Heavy metals

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

5.11 Pesticide residues

The contents of pesticide residues such as total BHC (benzene hexachloride), DDT (dichlorodiphenyltrichloroethane), aldrin, dieldrin and endrin shall be determined.

5.12 Sulfur dioxide

The content of sulfur dioxide should be determined.

6 Sampling

Sampling of *Poria cocos* sclerotium shall be in accordance with ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples of not less than 500 g shall be taken from each batch randomly, observed with the naked eye, smelled and tasted.

7.2 Microscopic identification

The testing method specified in ISO/TS 21310 shall apply.

7.3 Thin-layer chromatographic identification

See Annex A for additional information on thin-layer chromatographic identification.

7.4 Determination of moisture

The testing method specified in ISO 23723 shall apply.

7.5 Determination of total ash

The testing method specified in ISO 23723 shall apply.

7.6 Determination of extractives

The testing method specified in ISO 23723 shall apply.

7.7 Determination of pachymic acid

See Annex B for additional information on determination of pachymic acid.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues

The testing methods specified in ISO 22258 shall apply.

7.10 Determination of sulfur dioxide

The testing method specified in ISO 22590 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 used, with reference to this document;

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

The storage condition specified in ISO 22217 shall apply.

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

10 Marking and labelling

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

a) product name and Latin scientific name;

b) all quality features indicated in Clause 5, determined in accordance with methods specified in Clause 7;

c) gross weight and net weight of the product;

d) country and province or state of origin of the products;

e) date of production and expiry date of the products;

f) storage method;

g) any items required by the destination.

Annex A
(informative)

Thin-layer chromatographic identification

A.1 Preparation of test solution

Weigh 1 g of the powdered *Poria cocos* sclerotium through an 80-mesh or finer sieve, add 50 ml ethanol, ultrasonicate for 10 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of methanol as the test solution.

A.2 Preparation of reference solutions

a) Dissolve pachymic acid in methanol to prepare the reference solution containing 0,1 mg/ml.

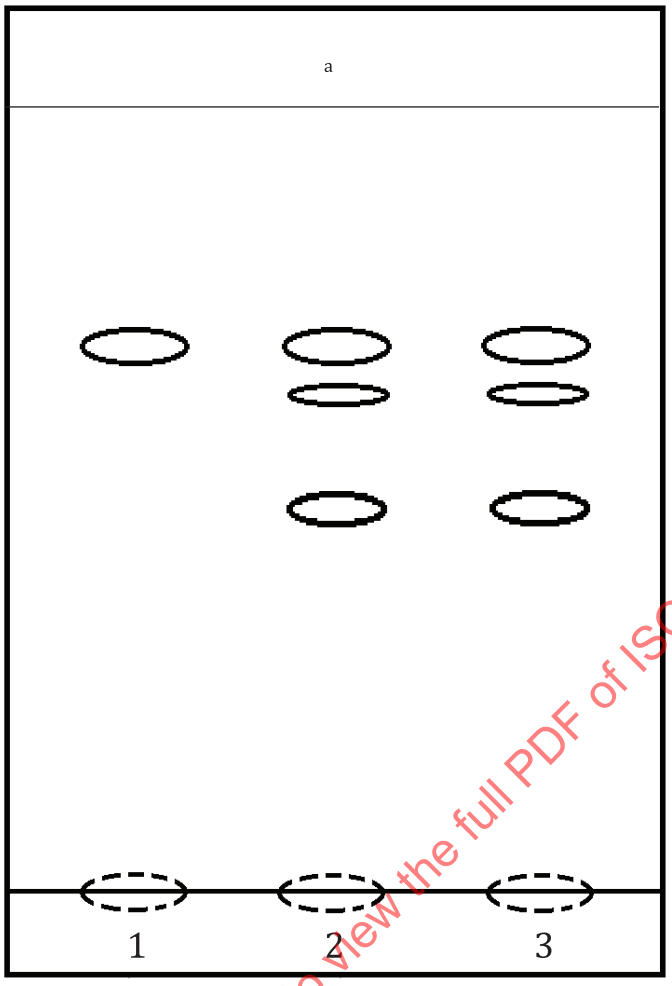
b) Prepare a solution of l g of poria reference drug in the same manner as in A.1 as the reference drug solution.

A.3 Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate and formic acid in the volume ratio of 7:3:0,2 as the mobile phase.

A.4 Identification by TLC

Apply the reference solution (2 µl), reference drug solution (2 µl) and test solution (2 µl) on the same silica gel GF_{254} TLC plate. Develop the plate with the mobile phase, take the plate out and dry in air, spray with a mixture of 5 % solution of vanillin in sulfuric acid and ethanol (1:4) and heat at 105 °C until the spots are clear. The main spots in the chromatogram obtained with the test solution correspond in position and colour to the spots obtained with the reference solutions. A typical reference TLC chromatogram is shown in Figure A.1.



Key

1 pachymic acid reference solution
2 reference drug solution of *Poria cocos* sclerotium
3 test solution of *Poria cocos* sclerotium
[a] Top of the plate.

Figure A.1 — Schematic diagram of typical TLC chromatogram of *Poria cocos* sclerotium

Annex B
(informative)

Determination of pachymic acid

B.1 Preparation of test solution

Weigh 2,0 g of the powdered *Poria cocos* sclerotium, through an 80-mesh or finer sieve, in a 50 ml stoppered conical flask. Add 20 ml of methanol. Apply ultrasonic treatment (powder: 500 W, rate: 40 kHz) for 30 min. Filter with filter paper, then extract the residue once again and combine the filtrate. Heat and condense to a small amount, then transfer to a 25 ml volumetric flask; add methanol to the mark and shake well. Filter through a 0,45 µm membrane filter and use the successive filtrate as the test solution.

B.2 Preparation of reference solution

Weigh a quantity of pachymic acid to a measuring flask then dissolve in methanol to prepare a solution containing 50 µg per ml as the reference solution.

B.3 Chromatographic system and high-performance liquid chromatography (HPLC) assay

B.3.1 Column

a) Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

b) Size: l = 0,25 m, $Ø$ = 4,6 mm, particle size = 5,0 µm.

c) Theoretical plates: not less than 10 000.

B.3.2 Mobile phase

a) Mobile phase A: acetonitrile.

b) Mobile phase B: 0,01 % phosphoric acid solution.

c) Programme of gradient elution (see Table B.1).

Table B.1 — Gradient programme

Time min	**Mobile phase A** volume fraction, %	**Mobile phase B** volume fraction, %
0	70	30
20	100	0

B.3.3 Flow rate

The flow rate is 1 ml/min.

B.3.4 Detector

The detector is 203 nm.

B.3.5 Column temperature

The column temperature is 25 °C.

B.3.6 Injection volume

The injection volume is 10 µl.

B.3.7 Content calculation of pachymic acid

Inject 10 µl each of the reference solution and the test solution into the column for chromatographic analysis and determine the content of pachymic acid in the test solution by the external standard method. The content of pachymic acid is calculated with Formula (B.1):

$$w = \frac{A_x \times C_s}{A_s \times 10^6} \times \frac{25}{m_s \times (1 - w_M)} \times 100 \quad \text{(B.1)}$$

where

A_x is the peak area of pachymic acid in the test solution;

A_s is the peak area of pachymic acid in the reference solution;

C_s is the concentration of pachymic acid in the reference solution;

m_s is the mass of the test sample;

w_M is the moisture of the test sample (%);

w is the content of pachymic acid (%).

B.3.8 Typical HPLC chromatogram

A typical reference HPLC chromatogram is shown in Figure B.1.