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Traditional Chinese medicine — *Codonopsis pilosula* root

Médecine traditionnelle chinoise — Racine de Codonopsis pilosula

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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

Codonopsis pilosula root is dried root of *Codonopsis pilosula* (Franch.) Nannf. (Family: Campanulaceae). It has a long history of use in East Asian countries for invigorating the stomach and spleen and benefitting qi.

Clinically, *Codonopsis pilosula* root is effective for the treatment of anaemia and malnutrition. To date, *Codonopsis pilosula* root occupies a huge share of the international market. However, many problems affect the quality of *Codonopsis pilosula* root, such as limited wild resources, increasing demand, the high investment cost of planting, different quality requirements among different countries and regions, and different packaging, transportation and storage conditions. Therefore, standardization of *Codonopsis pilosula* root is required.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.2, 5.3, 5.4, 5.5 and 5.7 in their national standards. Examples of national and regional values are given in Annex D.

Traditional Chinese medicine — *Codonopsis pilosula* root

1 Scope

This document specifies the minimum requirements and test methods for *Codonopsis pilosula* root derived from the plant of *Codonopsis pilosula* (Franch.) Nannf.

It is applicable to *Codonopsis pilosula* root that is sold as Chinese materia medica (whole medicinal materials) and decoction pieces derived from this plant.

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 1577, *Tea — Determination of acid-insoluble 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*

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 23723:2021, *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
***Codonopsis pilosula* root**
dried root of *Codonopsis pilosula* (Franch.) Nannf. in the family of Campanulaceaea

3.2
marker compound
chemical constituent within a medicinal herb that can be used to verify its quality

Note 1 to entry: Usually described as active ingredients or chemicals that confirm the correct botanical identity of the starting material.

Note 2 to entry: There may be one or more marker compounds for a medicinal herb.

4 Descriptions

Codonopsis pilosula root is dried root of *Codonopsis pilosula* (Franch.) Nannf. in the family of Campanulaceaea, as shown in Figure 1.

a) *Codonopsis pilosula* (Franch.) Nannf. **b) *Codonopsis pilosula* root**

Key
1 flower
2 plant
3 calyx
4 ovary
5 root
6 stem scars
7 lenticels

Figure 1 — Structure of *Codonopsis pilosula* root

5 Requirements

5.1 Morphological features

5.1.1 Appearance

The whole root is cylindrical, slightly curved. The root crown, which is broader than the root, forms an irregular head with a convex centre consisting of buds and numerous prominent, rounded verrucose stem scars (Figure 1, key 6). The whole root from the base to the central part shows deep longitudinal wrinkles and prominent scattered protuberances, resembling lenticels (Figure 1, key 7), over its entire length.

5.1.2 Colour

The external surface is brownish-yellow or brownish-grey. The colour of the transverse section is yellowish-white to light brown in the cortex, light yellow in the xylem, sometimes with a slit in the cortex. The cortex adheres tenaciously to the xylem and is difficult to remove.

5.1.3 Dimensions

The root is 10 cm to 35 cm in length measured from the base to the end of the root and 0,4 cm to 2 cm in diameter measured at the base of the root (Figure 1 b).

5.1.4 Texture

The texture is pliable and easily bendable or hard and easily breakable.

5.1.5 Odour and taste

The odour is slight and characteristic; the taste is slightly sweet.

5.2 Moisture

The moisture content in percentage mass should not be more than 16,0 %.

5.3 Total ash

The total ash content in percentage mass should not be more than 5,0 %.

5.4 Acid-insoluble ash

The total acid-insoluble ash content in percentage mass should not be more than 2,0 %.

5.5 Ethanol-soluble extractives

The ethanol-soluble extracts content in percentage mass should not be less than 35,0 %.

5.6 Thin-layer chromatogram (TLC) identification

The identification of marker compound, such as lobetyolin, with thin-layer chromatogram (TLC) shall present spots or bands obtained from the test and reference drug solution in the same position with the same colour.

5.7 Contents of marker compound

The content of the marker compound, such as lobetyolin, should be determined.

5.8 Heavy metals

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

5.9 Pesticide residues

The content of pesticide residues shall be determined.

5.10 Sulfur dioxide residues

The content of sulfur dioxide residues should be determined.

6 Sampling

Sampling of *Codonopsis pilosula* root shall be carried out in accordance with ISO 23723:2021, Clause 8.

7 Test methods

7.1 Macroscopic identification

Samples of not less than 500 g are taken from each batch randomly. These samples are examined by unaided visual inspection, smell and taste.

7.2 Determination of moisture content

The testing method specified in ISO 20409 applies.

7.3 Determination of total ash content

The testing method specified in ISO 1575 applies.

7.4 Determination of acid-insoluble ash content

The testing method specified in ISO 1577 applies.

7.5 Determination of ethanol-solution extractives content

See Annex A for additional information.

7.6 Thin-layer chromatogram (TLC) identification

See Annex B for additional information.

7.7 Determination of lobetyolin content

See Annex C for additional information.

7.8 Determination of heavy metals content

The testing method specified in ISO 18664 applies.

7.9 Determination of pesticide residues content

The testing method specified in ISO 22258 applies.

7.10 Determination of sulfur dioxide residues content

The testing method specified in ISO 22590 applies.

8 Test report

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

a) all the 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 might 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

Packaging should not transmit any odour or flavour to the product and should not contain substances which have the potential to damage the product or constitute a health risk. The packaging should be strong enough to withstand normal handling and transportation.

The storage requirements specified in ISO 22219 shall apply.

The *Codonopsis pilosula* root should be protected from light, moisture, pollution and the entry of foreign substances during long-distance delivery.

10 Marking and labelling

The general 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 mass or quantity;

d) contact information;

e) name of raw materials;

f) warning statements, if any;

g) expiry date;

h) storage method;

i) batch or lot number;

j) miscellaneous.

Annex A
(informative)

Determination of ethanol-soluble extractives

A.1 Determination of ethanol-soluble extractives

a) Weigh 250 g of sample to grind and pass it through a 24-mesh or coarse sieve. Dry the powder in a desiccator to constant weight. Weigh approximately 4 g of the dried powder into a 250-ml stoppered conical flask. Accurately add 100 ml of 45 % ethanol in water (volume fraction) and weigh.

b) Allow the mixture of the powder and 45 % ethanol to stand at room temperature for 18 hours, stir the mixture from time to time within the first 6 hours, then filter rapidly with a dry filter.

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

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

e) Calculate the mass fraction of ethanol-soluble extractives on the dried basis (%) with Formula (A.1).

$$M_{ese} = (M_1 - M_0) \times 4 / M_S \times 100\ \% \quad \text{(A.1)}$$

where:

M_{ese} is the mass fraction of ethanol-soluble extractives of *Codonopsis pilosula* root (%);

M_S is the mass of the sample (g);

M_0 is the mass of the evaporating dish (g);

M_1 is the mass of the evaporating dish and residue after drying (g).

Annex B
(informative)

Thin-layer chromatogram (TLC) identification

B.1 Preparation of test solution and reference solutions

a) Weigh 250 g of *Codonopsis pilosula* root to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 1,0 g of the powder, add 25 ml of methanol, sonicate for 30 minutes and filter. Evaporate the solution to dryness, then dissolve the residue with 2 ml of water and apply to a column (1,5 cm in inner diameter, 10 cm in length) packed with D101 macroporous resin. Elute with 50 ml of water and discard the water solution, then elute again with 50 ml of 50 % ethanol (volume fraction), collect the eluates and evaporate to dryness. Finally, dissolve the residue in 1 ml of methanol as the sample solution.

b) Weigh 250 g of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen or *Codonopsis tangshen* Oliv. powder and treat it in the same manner as in a) as the sample solutions of the other two species.

c) Dissolve a quantity of lobetyolin chemical reference substance (CRS) in ethanol to produce a solution containing 1 mg per ml as the reference solution.

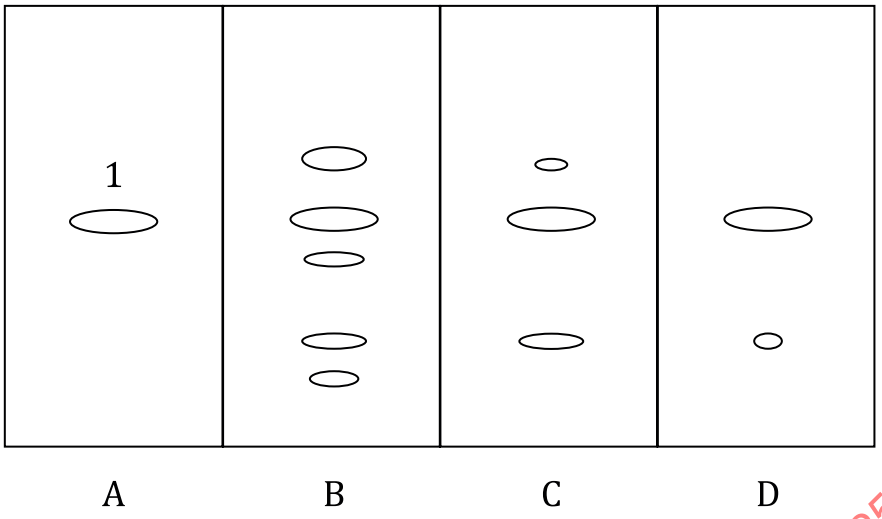
B.2 Developing solvent system

Prepare a mixture of *n*-butanol, glacial acetic acid and water in the volume ratio of 7:1:0,5 (volume fraction) as the mobile phase.

B.3 Procedure

Apply 5,0 µl each of the reference solution and test solution on the same TLC plate (silica gel binder) previously dried at 110 °C for 15 min in the oven. Develop the plate in the mobile phase, then remove it from the oven and dry in air. Examine the plate under ultraviolet light at 365 nm. Identify the spots of the test solution by comparing the positions and colours with these of the reference drug solution.

Typical reference TLC chromatograms are shown in Figure B.1



Key

1 lobetyolin
A lobetyolin reference solution
B *Codonopsis pilosula* root
C *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen
D *Codonopsis tangshen* Oliv.

Figure B.1 — Schematic diagram of typical TLC chromatogram of *Codonopsis* root

Annex C
(informative)

Determination of lobetyolin content

C.1 Preparation of reference standard solution

Dissolve a quantity of lobetyolin CRS with methanol 0,1 mol/l hydrochloric acid (1:1, volume fraction) in a brown volumetric flask to produce a solution containing 0,3 mg of each per ml as the reference solution.

C.2 Preparation of test solution

a) Weigh 250 g of *Codonopsis pilosula* root to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 3 g of the powder in a 100-ml round-bottomed flask. Accurately add 50 ml of methanol 0,1 mol/l hydrochloric acid (1:1, volume fraction) and sonicate for 1 hour. Cool and weigh again. Replenish the loss of solvent with methanol 0,1 mol/l hydrochloric acid and mix well, centrifuge at 3 500 rpm for 10 minutes, filter and use the successive filtrate. Filter the supernatant through a 0,22-µm millipore filter unit prior to the high-performance liquid chromatography (HPLC) analysis.

b) Weigh 250 g of *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen or *Codonopsis tangshen* Oliv. powder and treat it in the same manner as in a) as the sample solutions of the other two species.

C.3 Chromatographic system

C.3.1 Column

C.3.1.1 Stationary phase: octadecylsilance chemically bonded to porous silica particles, 5 µm in diameter as analysing column or equivalent.

C.3.1.2 size: l = 250 mm, Φ = 4,6 mm.

C.3.2 Mobile phase

C.3.2.1 Mobile phase A: 0,2 % (volume fraction) acetic acid in water for chromatography R.

C.3.2.2 Mobile phase B: acetonitrile for chromatography R.

C.3.2.3 Gradient elution: mixture of mobile phases A and B in gradient elution as shown in Table C.1.

Table C.1 — Mixture of mobile phases A and B in gradient elution

Time min	Mobile phase A %	Mobile phase B %
0 to 20	90 to 80	10 to 20
20 to 60	80 to 0	20 to 100

C.3.3 Flow rate: 1,0 ml/min.

C.3.4 Detector: 267 nm.

C.3.5 Column temperature: 40 °C.

C.3.6 Injection volume: 10 µl.

C.4 Content calculation of lobetyolin

C.4.1 The content of lobetyolin is calculated with Formula (C.1):

$$M_{\mathrm{LOB}}(\%)=\frac{C_{\mathrm{s}}\times 10^{-3}\times 100}{M\times(1-C_{\mathrm{m}})}\times 100\ \% \qquad \text{(C.1)}$$

where:

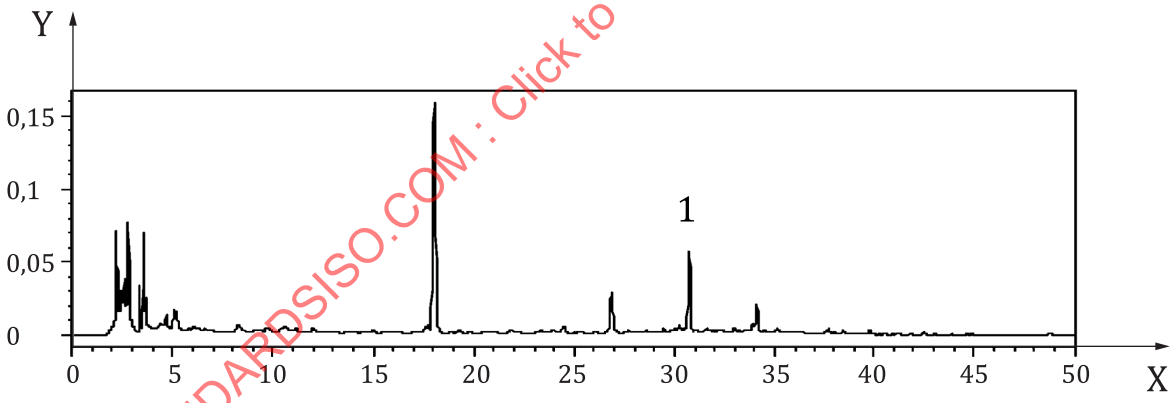
M_{LOB} is the content of lobetyolin (%);

C_{s} is the average content of the sample (mg/ml);

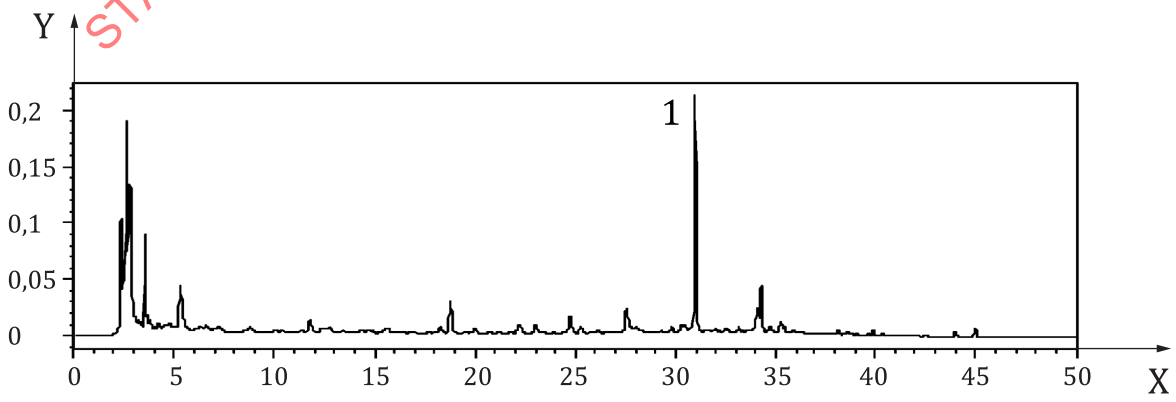
M is the mass of *Codonopsis pilosula* root taken to prepare the sample solution (g);

C_{m} is the moisture content of the sample (%).

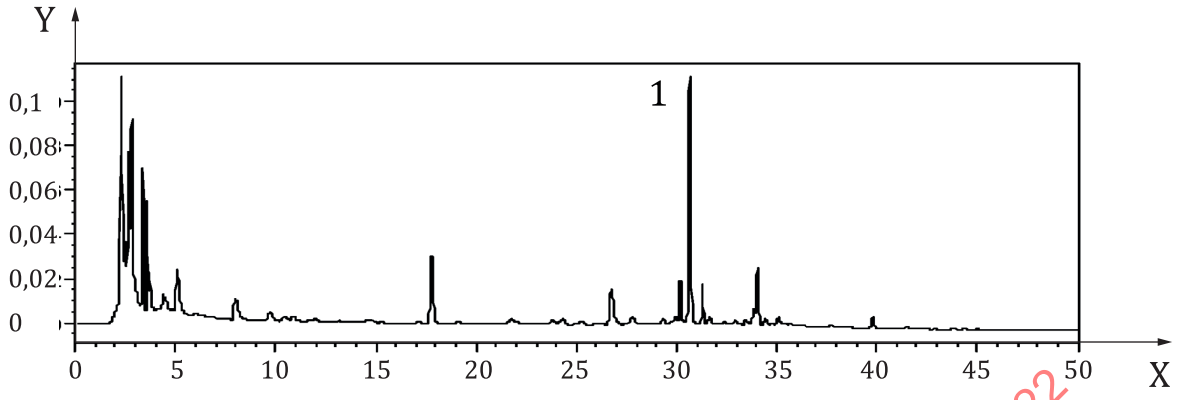
C.4.2 Typical reference HPLC chromatograms are shown in Figure C.1.



a) *Codonopsis pilosula* root



b) *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen



c) *Codonopsis tangshen* Oliv.

Key

X retention time (min)

Y absorbance unit

1 lobetyolin

Figure C.1 — Typical reference HPLC chromatograms of *Codonopsis* root