INTERNATIONAL STANDARD

ISO 23959

First edition
2021-08

Traditional Chinese medicine — *Glehnia littoralis* root

Médecine traditionnelle chinoise — racine de Glehnia littoralis

Reference number
ISO 23959:2021(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

Glehnia littoralis root is the dried or dried peeled root of *Glehnia littoralis* Fr. Schmidt ex Miq. (Apiaceae), which is recorded in the Chinese, Japanese and Korean pharmacopoeias, Hong Kong Chinese Materia Medica Standards and Taiwan herbal pharmacopoeia. *Glehnia littoralis* root is internationally recognized as a medicinal material and there is great demand for it in the international market. However, there are many problems seriously affecting the international trade of *Glehnia littoralis* root, including the following:

1) Quality requirements for *Glehnia littoralis* root are different among different countries and regions.

2) *Glehnia littoralis* root is often substituted with fake and inferior *Glehnia littoralis* root.

3) Different collecting times, processing methods, packaging, transportation and storage conditions often result in different qualities of *Glehnia littoralis* root.

Therefore, the establishment of an International Standard for *Glehnia littoralis* root is necessary to guarantee the quality, safety and consistency of this valuable herbal medicine. This document includes sections on morphology evaluation (general, macroscopic and microscopic characteristics), physicochemical indexes (moisture, total ash, acid-insoluble ash, marker compounds, pesticide residues and sulfur dioxide residue) and heavy metal (lead, arsenic, cadmium and mercury) content.

As national implementation may differ, national standards bodies are invited to modify the values given in 5.4, 5.5, and 5.6 in their national standards. Examples of national and regional values are given in Annex D.

Traditional Chinese medicine — *Glehnia littoralis* root

1 Scope

This document specifies the quality and safety requirements of *Glehnia littoralis* root, which is derived from the plant *Glehnia littoralis* Fr Schmidt ex Miq.

This document applies to *Glehnia littoralis* root that is sold and used as natural medicine in international trade, including 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 1666, *Starch — Determination of moisture content — Oven-drying method*

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

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*

World Health Organization, *Quality control methods for herbal materials,* 2011

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
tap root
main root of *Glehnia littoralis* Fr. Schmidt ex Miq. that grows straight downwards and produces smaller side roots

Note 1 to entry: See Figure 1, B.

3.2
stem scar
scar left on the tap root when the stem is removed

Note 1 to entry: See Figure 1, B.

3.3
root length
longest distance from the bottom to the stem scar of the tap root

Note 1 to entry: See Figure 1, B.

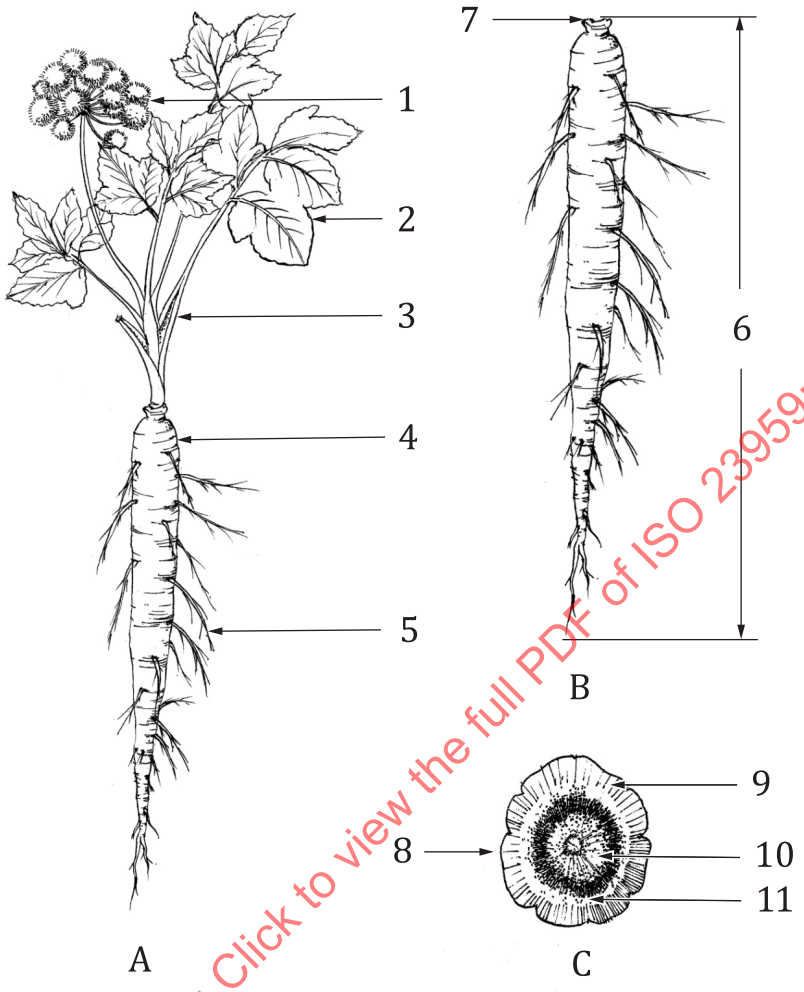
Note 2 to entry: Length is measured in centimetres.

3.4
batch
samples collected from the same particular place at the same time

[SOURCE: ISO 21317:2019, 3.5]

4 Descriptions

Glehnia littoralis root is the dried or dried peeled root of *Glehnia littoralis* Fr. Schmidt ex Miq. (Apiaceae), and the colour of its outer surface is pale yellowish-white to yellowish-brown (see Figure 1).



Key

A	plant of *Glehnia littoralis* Fr. Schmidt ex Miq.	5	lateral root
B	whole root of *Glehnia littoralis* Fr. Schmidt ex Miq.	6	tap root length
C	transverse section of the tap root	7	stem scar
1	inflorescence	8	epidermis
2	leaf	9	phloem
3	leaf sheath	10	xylem
4	tap root	11	cambium

Figure 1 — Structure of *Glehnia littoralis*

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling.

a) *Glehnia littoralis* root shall be clean and free from foreign matter.

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

5.2 Macroscopic characteristics

a) The root is slender-cylindrical. The top section of the root is slender, the greater portion of the middle section of the root is thick and the end section of the root becomes slender again towards the distal end (see Figure 1, A and B).

b) The top section of the root is often marked with remnants of yellowish-brown rhizome base.

c) The root is 9 cm to 45 cm long, 0,2 cm to 1,5 cm in diameter.

d) The outer surface is pale yellowish-white to yellowish-brown and somewhat rough. There are fine longitudinal wrinkles and grooves and brownish-yellow punctiform protuberance scars of rootlets across the surface of the root.

e) The bark (or cortex) section of the root's fracture is yellowish-white and the xylem section of the root's fracture is brown.

f) The texture is hard and fragile, and the root is easily broken.

g) The odour of the root is distinctively fragrant and the taste is slightly sweet.

5.3 Microscopic characteristics

5.3.1 Transverse section characteristics

a) Cortex consists of several rows of parenchyma cells.

b) Phloem is broad and has cleft. Phloem rays and groups of sieve tubes in the inner part are encrusted with densely arranged secretory canals. Secretory canals are 20 µm to 65 µm in diameter and contain yellowish-brown secretions. Each secretory canal is surrounded by 5 to 8 secretory cells.

c) Cambium is prominent and in a ring.

d) Xylem rays are broad and their width is 2 to 5 column cells. Vessels occur singly and are scattered or arranged in a V-shape configuration.

e) Parenchyma cells contain gelatinous starch masses.

5.3.2 Powder characteristics

a) The colour of the powder is yellowish-white.

b) Fragments of secretory canals containing yellowish-brown secretion are found frequently.

c) Yellowish-brown secretion and gelatinous starch masses are found frequently and their shape is irregular.

d) Vessel elements appear singly or in groups. The reticulate wall of the vessel elements is thick and the pits of the reticulate wall are long and wide.

e) Parenchyma cells are sub-rectangular and abundant.

5.4 Moisture

The content of moisture should not be more than 13,0 %.

5.5 Total ash

The content of total ash should not be more than 6,0 %.

5.6 Acid-insoluble ash

The content of acid-insoluble ash should not be more than 1,5 %.

5.7 Thin-layer chromatogram identification

The identification of *Glehnia littoralis* root with thin-layer chromatogram (TLC) shall present spot(s) of marker compound(s) such as falcarinol and other spots obtained from the test solution, reference standard solution and reference drug solution in the same positions with the same colour after evenly spraying the chromogenic agent.

5.8 Marker compounds

The content of marker compounds (e.g. polyacetylenes such as falcarinol) should be determined.

5.9 Heavy metals

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

5.10 Pesticide residues

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

5.11 Sulfur dioxide residue

The content of sulfur dioxide residue should be determined.

6 Sampling

Sampling of *Glehnia littoralis* root shall be in accordance with the World Health Organization's *Quality control methods for herbal materials*, 'General advice on sampling'.

a) From a batch of five containers or packaging units, take a sample from each one.

b) From a batch of between 6 and 50 units, take a sample from five units.

c) From a batch of over 50 units, sample 10 %, rounding up the number of units to the nearest multiple of 10. For example, a batch of 51 units would be sampled as 60 units; i.e. take samples from six packages.

d) From each selected container or package, take three original samples from the top, middle and bottom of the container or package. The three original samples should then be combined into a pooled sample that should be carefully mixed.

e) The average sample is obtained by quartering. Take some of the pooled sample, adequately mixed, place into an even, square-shaped heap, and divide this diagonally into four equal parts. Take two diagonally opposite parts and mix carefully.

f) Repeat the process as necessary until the required quantity, to within ±10 %, is obtained.

g) Using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material.

h) The final samples are tested for the measurement and analyses specified in Table 1. Ground samples shall be used in the measurement and analyses, except the identification of macroscopic characteristics. The test samples shall be finely ground, carefully mixed and sealed.

Table 1 — Maximum weight of batch and minimum weight of final sample

Maximum weight of batch kg	Minimum weight of final sample g		
	For analysis of marker compounds	For analysis of heavy metals	For other analyses
5 000	250	250	500
NOTE Other analyses include the identification of macroscopic and microscopic characteristics, the determination of moisture, total ash and acid-insoluble ash contents, and the TLC identification.			

7 Test methods

7.1 Macroscopic identification

Test samples of not less than 200 g shall be observed with the naked eye, smell and taste, and the length and diameter of the root measured.

7.2 Microscopic identification

7.2.1 Transverse section examination

See Annex A, A.1 for information.

7.2.2 Powder examination

See Annex A, A.2 for information.

7.3 Determination of moisture

The testing method specified in ISO 1666 shall apply.

7.4 Determination of total ash

The testing method specified in ISO 1575 shall apply.

7.5 Determination of acid-insoluble ash

The testing method specified in ISO 1577 shall apply.

7.6 TLC identification

See Annex B for information.

7.7 Determination of marker compounds

See Annex C for testing method of falcarinol.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues

The testing method specified in ISO 22258 shall apply.

7.10 Determination of sulfur dioxide residue

The testing method specified in ISO 22590 shall apply.

8 Test report

For each test, the test report shall specify at least 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, i.e. ISO 23959:2021;

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 have possibly 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 requirements of storage condition specified in ISO 22217 shall apply. The packaging and transportation shall not transmit any odour or flavour to the product and shall not contain substances which are liable to affect the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

Glehnia littoralis root shall be protected from light, moisture, pollution and the entry of foreign matter during long-distance delivery. Carriers should be well ventilated to keep the contents dry and free from moisture.

10 Marking and labelling

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

a) the product name and Latin scientific name of the original plant;

b) all quality features indicated in 5.2 to 5.9, determined in accordance with the methods specified in Clause 7;

c) the maximum weight of the batch and the minimum weight of samples specified in Table 1;

d) the country and province or state of origin of the product, as well as the name, trademark or logo of the producer and supplier;

e) the production date, batch number and expiry date of the product;

f) the storage method;

g) items required by the regulatory body of the destination country.

Annex A
(informative)

Microscopic identification

A.1 Transverse section examination

a) After softening with boiling water and 15 % hydrofluoric acid solution, cut the test sample into slices.

b) Place a slice 10 µm to 20 µm thick onto a glass microscope slide.

c) Place two or three drops of chloral hydrate solution on a glass microscope slide and then cover the preparation with a cover slip.

d) Examine under a microscope.

A.2 Powder examination

a) Pulverize the test sample and pass it through a 100-mesh or coarser sieve.

b) Place two or three drops of chloral hydrate solution on a glass microscope slide, disperse a very small quantity of the powdered test sample in the liquid and then cover the preparation with a cover slip.

c) Heat the preparation very gently to boiling on a hot plate or a micro gas burner.

d) Maintain gentle boiling for a short time and make sure that the quantity of mounting fluid is sufficient.

e) If necessary, add more fluid using a tapered glass pipette.

f) Allow to cool and then examine under a microscope.

g) Repeat the heating until the starch granules and the water-soluble contents of the cells are no longer visible.

h) Place one or two drops of dilute glycerin under the cover slip using a tapered glass pipette and then examine under a microscope.

Annex B
(informative)

TLC identification

B.1 Preparation of test solution

Weigh 3,0 g of the powdered test sample and place it into a suitable conical flask with stopper; add 30 ml of ethanol to the flask and extract the test sample ultrasonically for 30 min. After filtering, transfer the filtrate into a suitable round-bottomed flask. Evaporate the filtrate to dryness under reduced pressure. Dissolve the residue with 5 ml of methanol and then use the liquid as the test solution.

B.2 Preparation of reference solutions

a) Weigh 3,0 g of the powdered *Glehnia littoralis* root reference drug and then treat it in the same manner as the reference drug solution in B.1.

b) Dissolve 2,5 mg of reference substance falcarinol into 5 ml of methanol to obtain the reference standard solution containing 0,5 mg/ml of falcarinol.

B.3 Preparation of developing solvent

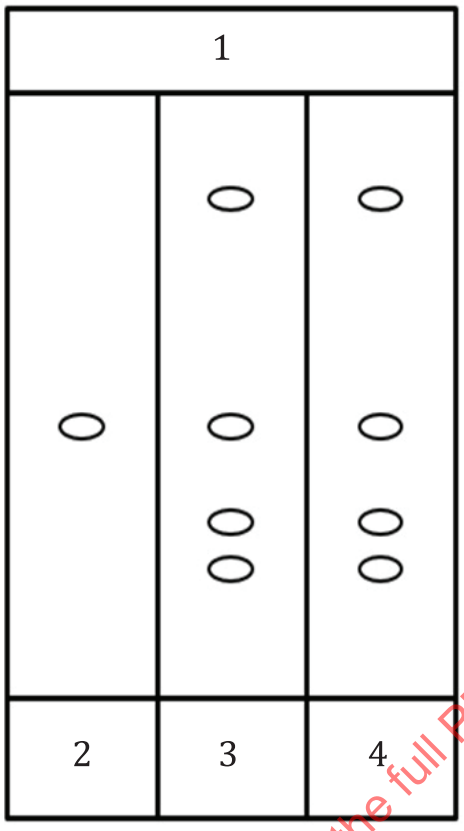
Prepare a mixture of petroleum ether (60 °C to 80 °C) and ethyl acetate in the volume ratio of 5:1 as the developing solvent.

B.4 Preparation of chromogenic agent

Slowly add 10 ml of concentrated sulfuric acid into 90 ml of ethanol to obtain the chromogenic agent.

B.5 Identification by TLC

Spot 5 µl of each of the three solutions in B.1 and B.2 on the same silica gel GF254 plate, previously dried in an oven at 105 °C for 30 min. Develop the plate with the developing solvent in B.3. Remove the plate and dry in air. Evenly spray the chromogenic agent in B.4 and dry the plate at 105 °C to make the spots clear. Identify the falcarinol spot and other spots of the test solution by comparing the positions (or retention values) and colour with those of the reference standard and reference drug solutions. A schematic diagram of typical reference TLC of *Glehnia littoralis* root is shown in Figure B.1.



Key

1	top of the plate	
2	reference standard solution	falcarinol
3	reference drug solution	*Glehnia littoralis* root reference drug
4	test solution	*Glehnia littoralis* root sample

Figure B.1 — Schematic diagram of typical reference TLC of *Glehnia littoralis* root

Annex C
(informative)

Determination of falcarinol

C.1 Preparation of test solution

Put about 2,0 g (accurate to 0,000 1 g) of the powdered test sample into a suitable round-bottomed flask. Accurately add 50 ml of methanol to the flask and then extract the test sample using the reflux extraction method for 60 min. After cooling to room temperature, filter and transfer the filtrate into another suitable round-bottomed flask. Repeat the extraction and combine the filtrate. Evaporate the filtrate to dryness under reduced pressure. Dissolve the residue with 5 ml of methanol, filter through a 0,45 µm filtration membrane and then use the liquid as the test solution.

C.2 Preparation of reference solution

Dissolve an appropriate amount of reference substance falcarinol into methanol to obtain the reference solution containing 0,2 mg of falcarinol in each millilitre.

C.3 Chromatographic system

C.3.1 Stationary phase of column: octadecylsilyl (ODS) chemically bonded porous silica particles, 5 µm in diameter as analysing column or equivalent.

C.3.2 Size of column: l = 0,25 m, $Ø$ = 4,6 mm.

C.3.3 Mobile phase: a mixture of acetonitrile and water in the volume ratio of 72:28.

C.3.4 Flow rate: 1,0 ml/min.

C.3.5 Detection wavelength: 205 nm.

C.3.6 Injection volume: 10 µl.

C.3.7 System suitability: minimum of 2500 theoretical plates, calculated for the peak due to falcarinol in the chromatogram.

C.4 Detection and content calculation

Accurately draw 10 µl of the reference solution and 10 µl of the test solution and perform detection under the conditions in C.3. A typical reference high performance liquid chromatography (HPLC) chromatogram of *Glehnia littoralis* root is shown in Figure C.1. Obtain the peak area of falcarinol using the automatic integration method. The content of falcarinol in *Glehnia littoralis* root, w_C, expressed as a percentage by mass of a sample, is calculated using Formula (C.1) (on the dried basis):

$$w_C = \frac{A_{\text{sam}} \times m_{\text{ref}} \times V_{\text{sam}} \times p_{\text{ref}} \times 100}{A_{\text{ref}} \times m_{\text{sam}} \times V_{\text{ref}} \times p_{\text{sam}} \times 1\,000} \tag{C.1}$$

where