



**International
Standard**

ISO 9299

**Traditional Chinese medicine —
Curcuma longa rhizome**

Médecine traditionnelle chinoise — Rhizome de Curcuma longa

**First edition
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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 document 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 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

The medicinal history of *Curcuma longa* rhizome dates back 4 000 years. *Curcuma longa* rhizome has been used as a traditional herbal medicine in China, India, Indonesia, Japan, Malaysia, South Korea, Thailand, and other countries. It has been used for the treatment of digestive, respiratory and circulatory diseases, as well as skin diseases.

The establishment of an international standard for *Curcuma longa* rhizome is necessary to guarantee the clinical effectiveness, safety and controllability in global commerce and trade.

As national implementation may differ, national standards bodies are invited to modify the values given in [5.6](#), [5.7](#), [5.8](#), [5.9](#), [5.10](#) and [5.11](#) in their national standards. Examples of national and regional values are given in [Annex C](#). In addition, examples for traditional grading of *Curcuma longa* rhizome are given in [Annex D](#).

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Traditional Chinese medicine — *Curcuma longa* rhizome

1 Scope

This document specifies the quality and safety requirements for *Curcuma longa* rhizome.

This document applies to the production and sale of cultivated *Curcuma longa* rhizome that is sold and used as natural medicine in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces derived from this rhizome.

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 22283, *Traditional Chinese medicine — Determination of aflatoxins in natural products by LC-FLD*

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

***Curcuma longa* rhizome**

dried rhizome of *Curcuma longa* L. (Fam, Zingiberaceae) after primary processing (3.2)

Note 1 to entry: *Curcuma longa* rhizome is harvested when the aerial parts of the plant are withered, e.g. from December to the next February in China.

3.2

primary processing

stage of the pre-treatment of natural materials during the collecting and harvesting process by which the raw materials are transformed into medicinal materials

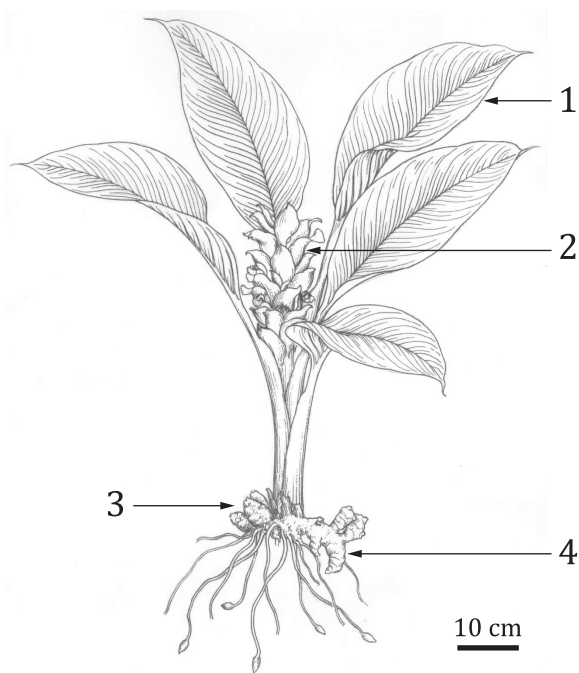
Note 1 to entry: The primary processing of *Curcuma longa* rhizome includes removing the fibrous roots, washing, boiling or steaming to the core, and drying in the sun or dry at the temperature below 55 °C.

[SOURCE: ISO 21300:2019, 3.4, modified — Note 1 to entry has been added.]

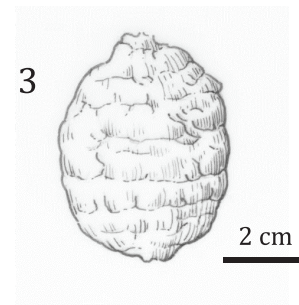
4 Descriptions

[Figure 1](#) illustrates the structure of *Curcuma longa* L.

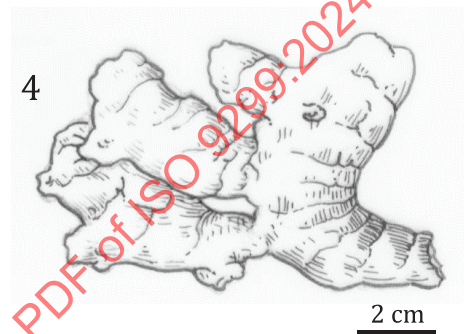
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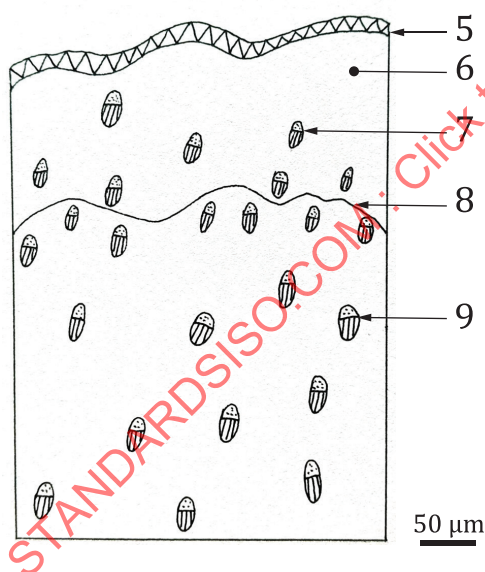
a) Plant of *Curcuma longa* L.



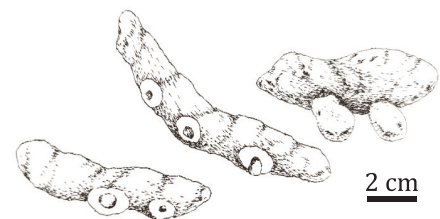
b) Main rhizome of *Curcuma longa* L.



c) Lateral rhizome of *Curcuma longa* L.



f) Sketch of transverse section of *Curcuma longa* L.



d) Rhizome of *Curcuma longa* L. (dried and unsliced)



e) Rhizome of *Curcuma longa* L. (decoction pieces)

Key

- 1 *Curcuma longa* leaf
- 2 *Curcuma longa* inflorescence
- 3 curcuma main rhizome
- 4 curcuma lateral rhizome
- 5 cork
- 6 cortex

- 7 leave-trace vascular bundle
- 8 endodermis
- 9 vascular bundle

Figure 1 — Structure of *Curcuma longa* L.

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling:

- a) The sample shall be clean and free from rootlets;
- b) The presence of living insects, mouldy root and rhizome, and external contaminants which are visible to the naked eye shall not be permitted.

5.2 Macroscopic features

5.2.1 The rhizomes are irregular ovoid, cylindrical or spindle-shaped, often curved, some with short forked branches. The outer surface is rough, with crinkled texture and distinct links, with rounded branch marks and fibrous root marks. The surface colour is deep yellow to yellowish brown.

5.2.2 The odour is fragrant. The taste is bitter and pungent.

5.2.3 The section feature is brown yellow to golden yellow, with horny appearance and wax-like lustre. The section colour is golden yellow to tan.

5.3 Microscopic feature

Examine the transverse section under a microscope. Epidermal cells are flattened and thin-walled. The cork is composed of several layers of cells, flattened and thin-walled, regularly arranged. A few leaf-trace vascular bundles are scattered across the cortex. The endodermis is distinct. The stele is broad; collateral vascular bundles are mostly scattered near the pericycle, gradually decreased inwards. See [Figure 1](#) F.

5.4 Thin-layer chromatography (TLC) features

The identification of extract by thin-layer chromatography (TLC) shall present the spots or bands with the same colour and position corresponding to those of reference solutions.

5.5 Marker compounds

The mass fractions of marker compounds, such as bisdemethoxycurcumin, curcumin and demethoxycurcumin, should be determined.

5.6 Essential oil

The mass fraction of essential oil should not be less than 4,0 %.

5.7 Ethanol-soluble extractive

The mass fraction of ethanol-soluble extractive should not be less than 6,0 %.

5.8 Total ash

The mass fraction of total ash should not be more than 8,0 %.

5.9 Acid-insoluble ash

The mass fraction of acid-insoluble ash should not be more than 1,0 %.

5.10 Moisture or loss on drying

The mass fraction of moisture should not be more than 12,0 % (azeotropic method) or the loss on drying should not be more than 17,0 %.

NOTE The azeotropic method is toluene distillation.

5.11 Foreign matter

The mass fraction of foreign matter should not be more than 3,0 %.

5.12 Heavy metals

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

5.13 Pesticide residues

The mass fractions of pesticide residues, such as total DDT, dieldrin, total BHC and aldrin, should be determined.

5.14 Sulfur dioxide

The mass fraction of sulfur dioxide should be determined.

5.15 Aflatoxins

The mass fractions of aflatoxin B1 and total aflatoxins (B1, B2, G1 and G4) should be determined.

6 Sampling

The sampling methods specified in ISO 23723 shall apply.

7 Test methods

7.1 Identification of macroscopic features

Samples of not less than 500 g are taken from each batch randomly. These samples are examined by visual observation in daylight, smelled and tasted.

7.2 Identification of microscopic features

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

7.3 Identification of thin-layer chromatogram

See [Annex A](#) for additional information on the identification of thin-layer chromatogram.

7.4 Determination of marker compounds

See [Annex B](#) for additional information on the determination of total content of curcuminoids.

7.5 Determination of essential oil

The test method specified in ISO 23723 shall apply.

7.6 Determination of ethanol-soluble extractive

The test method of ethanol-soluble extractive specified in ISO 23723 shall apply.

7.7 Determination of total ash

The test method of total ash specified in ISO 23723 shall apply.

7.8 Determination of acid-insoluble ash

The test method of acid-insoluble ash specified in ISO 23723 shall apply.

7.9 Determination of moisture

The azeotropic method (toluene distillation) and the test method of loss on drying (gravimetric determination) specified in ISO 23723 shall apply.

7.10 Determination of foreign matter

The test method specified in ISO 23723 shall apply.

7.11 Determination of heavy metals

The test method specified in ISO 18664 shall apply.

7.12 Determination of pesticide residues

The test method specified in ISO 22258 shall apply.

7.13 Determination of sulfur dioxide

The test method specified in ISO 22590 shall apply.

7.14 Determination of aflatoxin

The test method specified in ISO 22283 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 can 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 can damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The storage conditions specified in ISO 22217 shall apply.

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

10 Marking and labelling

The following items shall be marked or labelled on the packages in accordance with the method specified in ISO 21371:

- a) all quality features indicated in [Clause 5](#), determined in accordance with methods specified in [Clause 7](#);
- b) cross mass and net mass of the package;
- c) country and province/state of origin of the products;
- d) date of production, batch number and expiry date of the products;
- e) storage and transportation method.

Annex A (informative)

Identification of thin-layer chromatogram

A.1 Apparatus

Prepare the following apparatus: capillary tube, thin layer plate.

A.2 Procedure of identification

The procedure for the identification of curcumin in the ethanol extract of *Curcuma longa* rhizome is as follows.

- a) Weigh 0,2 g of powdered test sample and herbal reference drug, respectively, and pass it through an 80 mesh. Add 20 ml of absolute ethanol, shake, leave it for 30 min and filter. Evaporate the filtrate, the residue is dissolved in 2 ml of absolute ethanol as the test solution.
- b) Dissolve the chemical reference standard of curcumin in absolute ethanol to produce a solution containing 0,5 mg of curcumin per ml as the reference solution.
- c) Carry out the method for thin layer chromatography using silica gel TLC-plate, a mixture of chloroform, methanol and formic acid (96:4:0,7) as the mobile phase. Apply 4 µl of curcumin reference solution, herbal reference drug solution and test solution separately to the silica gel G plate.
- d) After developing and removal of the plate, dry the plate in air. Examine macroscopic features under sunlight and ultraviolet light (main wavelength: 365 nm).
- e) The same green-yellow fluorescent spots are shown in both chromatograms obtained with the test solution and the reference solution, confirming the presence of curcumin in the rhizome extract being tested.

The identification of ethanol extract of *Curcuma longa* rhizome comparing with the reference drug is shown in [Figure A.1](#).

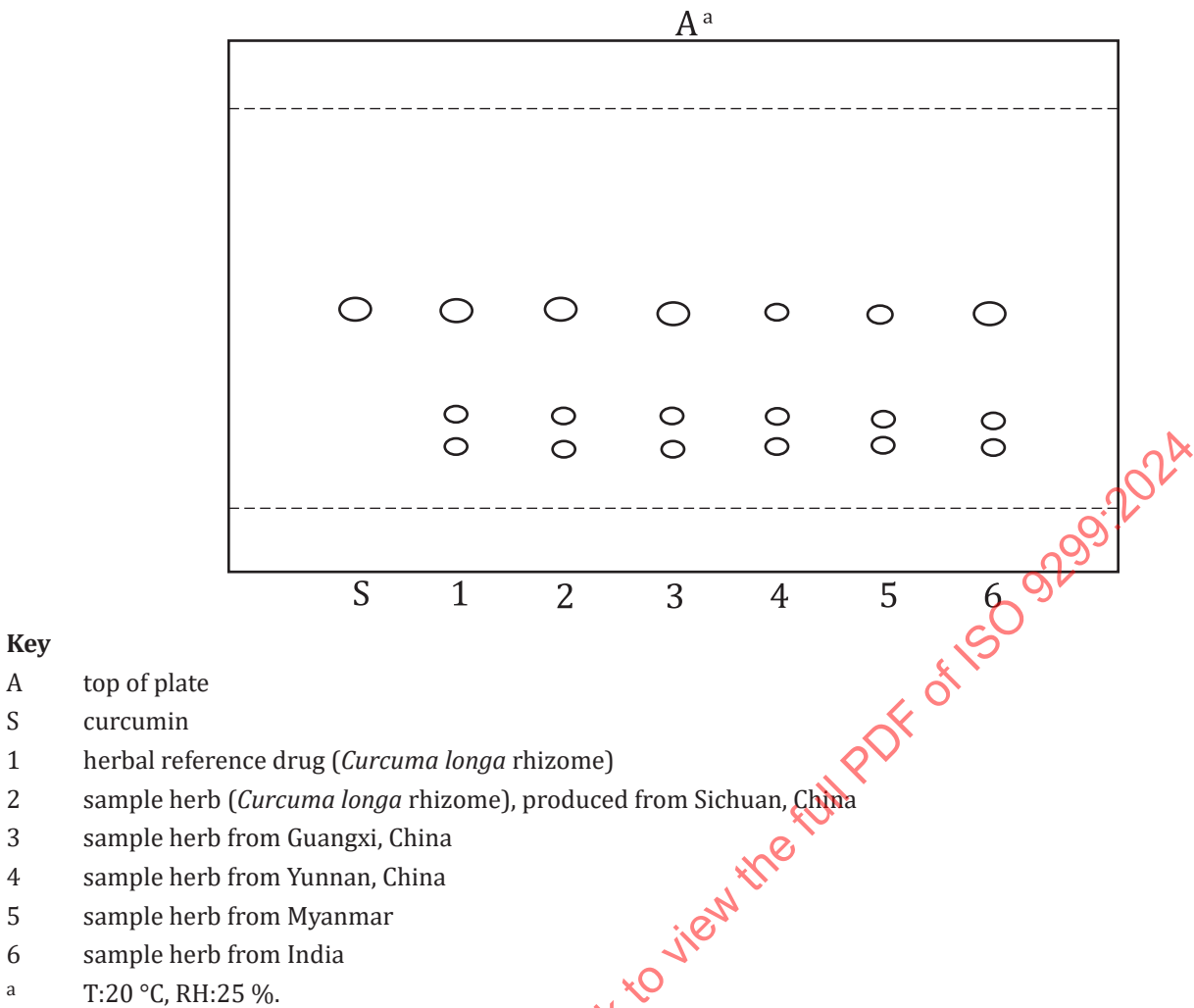


Figure A.1 — Identification of *Curcuma longa* rhizome by thin-layer chromatography

Annex B (informative)

Determination of total content of curcuminoids

B.1 Apparatus

Prepare the following apparatus:

- a) HPLC instrument;
- b) vortex;
- c) electronic analytical balance (one part per 100 000);
- d) electric thermostatic blast drying box.

B.2 Reagent

Prepare the following reagent:

- a) curcumin;
- b) demethoxycurcumin;
- c) bisdemethoxycurcumin;
- d) methanol;
- e) glacial acetic acid.

B.3 Preparation of test

B.3.1 Preparation for reference solution

Weigh 10 mg of curcumin for assay, and dissolve in methanol to make 50 ml of solution. Pipet 10 ml of this solution to a 50 ml volumetric flask, add methanol to the scale line and use this solution as the reference solution.

B.3.2 Preparation for sample solution

Weigh 200 mg of pulverized turmeric (80 mesh), add 25 ml of a mixture of methanol and glacial acetic acid (99:1), shake for 20 min, centrifuge, and separate the supernatant liquid. To the residue, add 25 ml of a mixture of methanol and glacial acetic acid (99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make 50 ml of solution, and use this solution as the sample solution.

B.3.3 Chromatographic system condition

Liquid chromatograph:

- a) octadecyl silane bonded silica gel as filler (4,6 × 150 mm, 5 µm);
- b) mobile phase: water-acetonitrile-glacial acetic acid (56:43:1);

- c) detection wavelength: 245 nm;
- d) flow rate: 1,0 ml/min.

B.3.4 System suitability

Dissolve 1 mg each of curcumin for assay, demethoxycurcumin and bisdemethoxycurcumin in methanol to make 5 ml of solution. When the procedure is run with 10 µl of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcumin are eluted in this order with the resolution among these peaks being not less than 1,5.

When the test is repeated 6 times with 10 µl of the reference solution under the operating conditions above, the relative standard deviation of curcumin is not more than 1,5 %.

B.4 Procedure of measurement

Inject 10 µl each of the sample solution and reference solution into the liquid chromatographic column for determination, and determine the peak areas, A_{TC} , A_{TD} and A_{TB} of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A_S of curcumin in the reference solution.

B.5 Expression of results

The amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) is calculated as follows.

$$\frac{M_S \times (A_{TC} + A_{TD} + A_{TB} \times 0,69)}{A_S} \times \frac{1}{5}$$

where

- M_S is the amount (mg) of curcumin for assay;
- A_{TC} is the peak area of curcumin in the sample solution;
- A_{TD} is the peak area of demethoxycurcumin in the sample solution;
- A_{TB} is the peak area of bisdesmethoxycurcumin in the sample solution;
- A_S is the peak area of curcumin in the reference solution;
- 1/5 is the dilution factor.

The relative correlation factors of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin to curcumin are 1,00, 1,00 and 0,69, respectively.

The sample contains not less than 1,5 % of the total content of bisdemethoxycurcumin ($C_{19}H_{16}O_4$), curcumin ($C_{21}H_{20}O_6$) and demethoxycurcumin ($C_{20}H_{18}O_5$), calculated on the basis of dried material.