INTERNATIONAL STANDARD

ISO 22212

First edition
2019-03

Traditional Chinese medicine — *Gastrodia elata* tuber

Médecine traditionnelle chinoise — Tubercule de Gastrodia elata

Reference number
ISO 22212:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Descriptions **2**
5 Requirements **4**
5.1 General characteristics 4
5.2 Morphological features 4
5.3 Moisture 4
5.4 Total ash 4
5.5 Dilute ethanol-soluble extract 4
5.6 Identification of marker compound(s) 4
5.7 Content of marker compound(s) 4
5.8 Heavy metals 4
5.9 Pesticide residues 5
5.10 Sulfur dioxide 5
6 Sampling **5**
7 Test methods **5**
7.1 Macroscopic identification 5
7.2 Determination of moisture content 5
7.3 Determination of total ash content 6
7.4 Determination of dilute ethanol-soluble extract content 6
7.5 Identification of marker compound(s) 6
7.6 Determination of marker compound(s) content 6
7.7 Determination of heavy metals 6
7.8 Determination of pesticide residues 6
7.9 Determination of sulfur dioxide content 6
8 Test report **6**
9 Packaging, storage and transportation **6**
10 Marking and labelling **7**
Annex A (informative) **Determination of moisture content** **8**
Annex B (informative) **Determination of dilute ethanol-soluble extract content** **9**
Annex C (informative) **Identification of gastrodin** **10**
Annex D (informative) **Identification of *p*-hydroxybenzyl alcohol** **12**
Annex E (informative) **Determination of gastrodin and *p*-hydroxybenzyl alcohol contents** **14**
Annex F (informative) **Reference values of national and regional limits in *Gastrodia elata* tuber** **17**
Bibliography **18**

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

Gastrodia elata tuber, the dried tuber of *Gastrodia elata* Bl. (Orchidaceae) after it has been steamed thoroughly, is a medicinal herb which has been used as an anticonvulsant, analgesic and sedative to treat general paralysis, epilepsy, tetanus and vertigo in Asian countries for thousands of years.

There are at least 16 countries and regions using *Gastrodia elata* tuber and its products. Major users include China, Japan, South Korea, the United State, Australia, Austria and Singapore. Due to its great demand and high price in the global market, trade in *Gastrodia elata* tuber has been complicated by adulteration, substitution and species identification issues. The toxic roots of other species, such as the plants of the Phytolaccaceae family, are sometimes misused as *Gastrodia elata* tuber, which can cause health risks. Factors including contamination, packaging and storage conditions also affect the quality of *Gastrodia elata* tuber.

The establishment of an international standard for *Gastrodia elata* tuber is therefore necessary to support its quality consistency, clinical effectiveness and safety in international trade.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.3, 5.4, 5.5 and 5.7 in their national standards. Examples of national and regional values are given in Annex F.

Traditional Chinese medicine — *Gastrodia elata* tuber

1 Scope

This document specifies minimum requirements and test methods for *Gastrodia elata* tuber that is derived from cultivated and artificially propagated *Gastrodia elata* Bl.

It is applicable to *Gastrodia elata* tuber that is sold and used as Chinese materia medica, specifically excluding the wild forms of the species.

2 Normative references

ISO 1575, *Tea — Determination of total ash*

ISO 5379, *Starches and derived products — Determination of sulfur dioxide content — Acidimetric method and nephelometric 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*

CODEX STAN 229-1993, REV.1-2003, *Analysis of pesticide residues: Recommended methods*

CAC/MRL01-2009, *Maximum Residue Limits for Pesticides in Foods*

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

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
***Gastrodia elata* tuber**
dried tuber of *Gastrodia elata* Bl. (Orchidaceae) after it has been steamed or boiled thoroughly

3.2
bud
undeveloped or embryonic shoot in red-brown to dark brown, which is parrot-beak-shaped and grows on the apex of the tuber

Note 1 to entry: See Figure 1.

3.3
latent bud
bud which remains undeveloped or dormant, arranged along the body of *Gastrodia elata* tuber

Note 1 to entry: See Figure 1.

3.4
sample
portion taken from the *batch* (3.5) during one single sampling action

3.5
batch
samples (3.4) collected from the same particular place at the same time

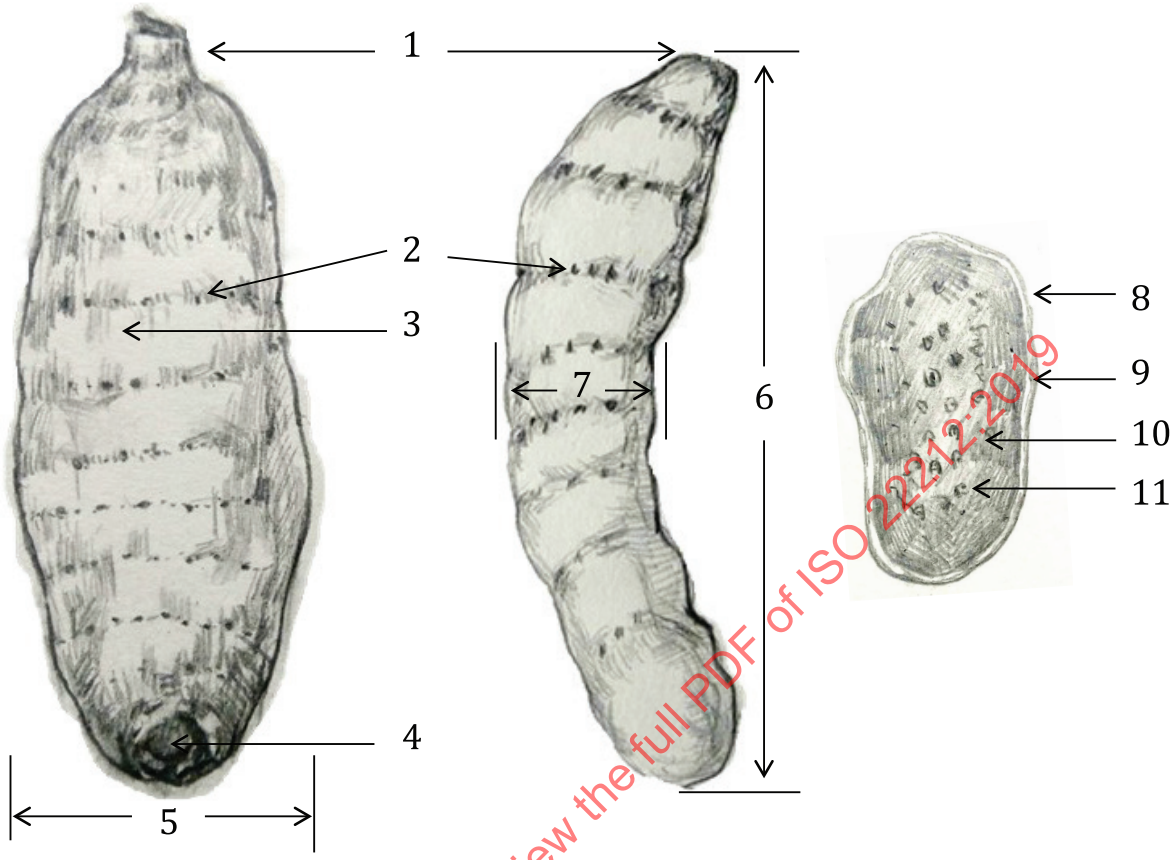
Note 1 to entry: This is not more than 5 000 kg.

3.6
final sample
samples (3.4) for the test required in this standard

Note 1 to entry: Final samples may be packed in different materials meeting conditions for specific tests (e.g. moisture or total ash).

4 Descriptions

Gastrodia elata tuber is the dried tuber of *Gastrodia elata* Bl. (Orchidaceae) after it has been steamed or boiled thoroughly, as shown in Figure 1.



Key

A	front view
B	lateral view
C	cross-sectional view
1	bud or remain of stem
2	latent bud
3	longitudinal wrinkle
4	rounded scar
5	tuber width
6	tuber length
7	tuber thickness
8	epidermis
9	hypodermis
10	stele
11	vascular bundle

Figure 1 — Structure of *Gastrodia elata* tuber

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Gastrodia elata* tuber shall be clean and free from foreign matter;

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

5.2 Morphological features

a) The tuber is ellipsoid or slat-shaped, slightly compressed, shrunken and somewhat curved.

b) The tuber is 3 cm to 15 cm long, 1,5 cm to 6 cm wide, and 0,5 cm to 4 cm thick.

c) The tuber mass is no less than 12 g.

d) The outer surface is yellowish-white to pale yellowish-brown, with longitudinal wrinkles and many transverse annulations arranged along latent buds. The brown thread is sometimes visible. There are reddish-brown to deep brown parrot-beak-shaped buds or remains of stem on the apex. There is a rounded scar at the lower end of the tuber.

e) The texture is hard and uneasily broken.

f) The fracture is fairly even, yellowish-white to brownish, and horny.

g) The odour is slight, and the taste is sweetish.

5.3 Moisture

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

5.4 Total ash

The mass fraction of total ash should not be more than 4,5 %.

5.5 Dilute ethanol-soluble extract

The mass fraction of dilute ethanol-soluble extract should not be less than 15,0 %.

5.6 Identification of marker compound(s)

The identification of marker compound(s), such as gastrodin or *p*-hydroxybenzyl alcohol, with thin-layer chromatography (TLC) shall present the spots or brands obtained from the test and reference solutions in the same position with the same colour.

5.7 Content of marker compound(s)

The content of marker compound(s) should be determined. For example, the sum of the mass fraction of gastrodin and *p*-hydroxybenzyl alcohol should not be less than 0,25 %.

5.8 Heavy metals

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

5.9 Pesticide residues

The contents of pesticide residues including Benzex, DDT and quintozene shall be determined.

5.10 Sulfur dioxide

The content of sulfur dioxide shall be determined.

6 Sampling

Sampling of *Gastrodia elata* tuber shall be done with reference to the World Health Organization 2011, *Quality control methods for herbal materials*.

a) Take a sample from each of a batch of five containers or packaging units.

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

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 for 60, i.e. take samples from six packages.

d) From each container or package selected, 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 mixed carefully.

e) The average sample is obtained by quartering. From the pooled sample, adequately mix into an even and square-shaped heap, and divide it 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.

Table 1 — Maximum mass of batch and minimum mass of the final sample

Maximum mass of tuber per batch kg	Minimum mass of final sample g		
	For macroscopic identification	For determination of marker compound(s) content	For other analyses
5 000	500	250	250
NOTE 1 The requirements are based on samples collected from different production regions of *Gastrodia elata* tuber.			
NOTE 2 Other analyses include the identification of marker compound(s) and the determination of moisture content, total ash, ethanol-soluble extractives, heavy metals, pesticide residues and sulfur dioxide.			

7 Test methods

7.1 Macroscopic identification

Samples of not less than 500 g are taken from each batch randomly and observed with the naked eye, smelt and tasted with the tongue.

7.2 Determination of moisture content

The testing method refers to Annex A for additional information.

7.3 Determination of total ash content

The testing method specified in ISO 1575 applies.

7.4 Determination of dilute ethanol-soluble extract content

The testing method refers to Annex B for additional information.

7.5 Identification of marker compound(s)

The testing methods refer to Annex C and Annex D for additional information.

7.6 Determination of marker compound(s) content

The testing method refers to Annex E for additional information.

7.7 Determination of heavy metals

The testing method specified in ISO 18664 applies.

7.8 Determination of pesticide residues

The testing methods specified in CAC/MRL01-2009 and CODEX STAN 229-1993, REV.1-2003 apply.

7.9 Determination of sulfur dioxide content

The testing method specified in ISO 5379 applies.

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, 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

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

The temperature for *Gastrodia elata* tuber storage shall be not higher than 20 °C. The storage time for *Gastrodia elata* tuber shall not exceed 36 months.

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

10 Marking and labelling

The labelling method specified in ISO 21371 applies. The following items shall be marked or 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 mass and net mass of the products;

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

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

f) storage and transportation method;

g) any items required by regulatory bodies of the destination country;

h) any other information requested by the buyer, such as the harvest year and packaging date (if known);

i) certificate information of artificial cultivation according to regulations of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora).

Annex A
(informative)

Determination of moisture content

Moisture content in *Gastrodia elata* tuber can be determined by the oven drying method.

Determination can be conducted in terms of the following steps:

a) Weigh 2 g to 5 g of sample powder and place it in a dried flat weighing bottle, the thickness of which is not more than 5 mm. The thickness of the sample powder shall not be more than 10 mm. Accurately weigh the bottle and sample.

b) Dry the bottle at 100 °C to 105 °C for 5 h with the bottle cap opened. Cover the cap and transfer the bottle into the dryer to cool for 30 min. Accurately weigh the bottle and sample.

c) Continue drying the bottle at the temperature in b) for 1 h. Cool the bottle and weigh it, until the mass difference of two successive weighings is not more than 5 mg.

d) According to the mass loss, calculate the moisture content of samples (%), C_m, with Formula (A.1):

$$C_m = (W_0 - W_1)/S \times 100\ \% \quad \text{(A.1)}$$

where

S is the mass of the sample before drying (g);

W_0 is the mass of the flat weighing bottle and sample before drying (g);

W_1 is the mass of the flat weighing bottle and sample after drying (g).

Annex B
(informative)

Determination of dilute ethanol-soluble extract content

Dilute ethanol-soluble extract content in *Gastrodia elata* tuber can be determined by the hot-dip method.

Determination can be conducted using the following steps.

a) Weigh 250 g of the sample to grind and pass it through a sieve of 24 mesh or a coarse sieve. Weigh approximately 0,5 g of the powder into a 250 ml stopper conical flask. Accurately add 50 ml ethanol. 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 mass with ethanol, mix well and filter.

c) Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into an 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 extract rapidly and accurately.

e) Calculate the percentage of dilute ethanol-soluble extract on the dried basis (%), C_e, with Formula (B.1):

$$C_e = (W_1 - W_0) \times 2 / S \times 100 \quad \text{(B.1)}$$

where

S is the mass of the sample (g);

W_1 is the mass of the evaporating dish and residue after drying (g);

W_0 is the mass of the evaporating dish (g).

Annex C
(informative)

Identification of gastrodin

C.1 Preparation of test solution

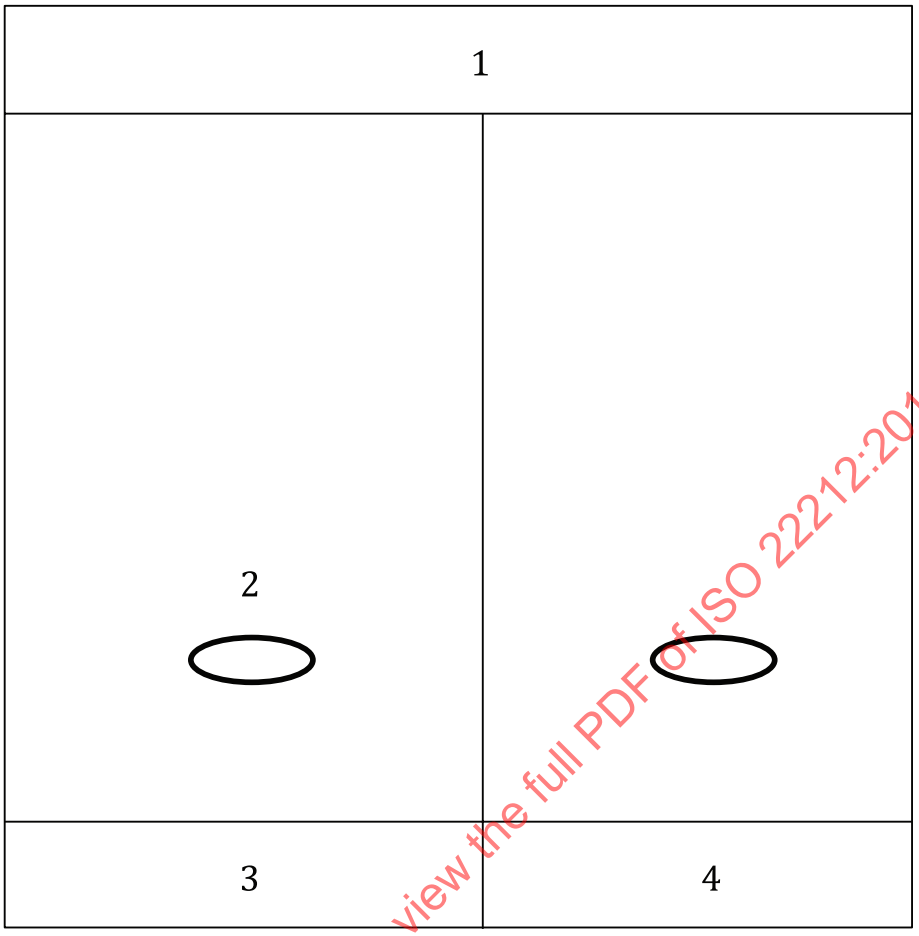
Weigh 0,5 g of the powdered sample and place it into a 25 ml stopper conical flask, then add 5 ml of 70 % methanol. Sonicate the mixture for 30 min. Filter the mixture and take the filtrate as the test solution.

C.2 Preparation of standard solution

Dissolve gastrodin in methanol to prepare the standard solution of 1 mg/ml.

C.3 Thin-layer chromatography (TLC) identification

Apply 5 µl of the standard solution and 10 µl of test solution on the same TLC plate (silica gel) previously dried at 110 °C for 15 min in the oven. Develop with a solution of a mixture of ethyl acetate, methanol and water in a volume fraction of 9:1:0,2 to a distance of about 10 cm below 10 °C. Take the plate out and dry in air. Spray 10 % phosphomolybdic acid-ethanol solution over the TLC plate and heat at 105 °C until the colour looks clear. Identify the gastrodin spot of the test solution by comparing the position and colour with those of the standard solution. A typical TLC chromatogram is shown in Figure C.1. A blue-purple spot appears at an *R*f value of about 0,15.



Key

1 top of the plate
2 gastrodin
3 standard solution
4 test solution

Figure C.1 — Schematic diagram of typical TLC chromatogram of gastrodin in *Gastrodia elata* tuber

Annex D
(informative)

Identification of *p*-hydroxybenzyl alcohol

D.1 Preparation of test solution

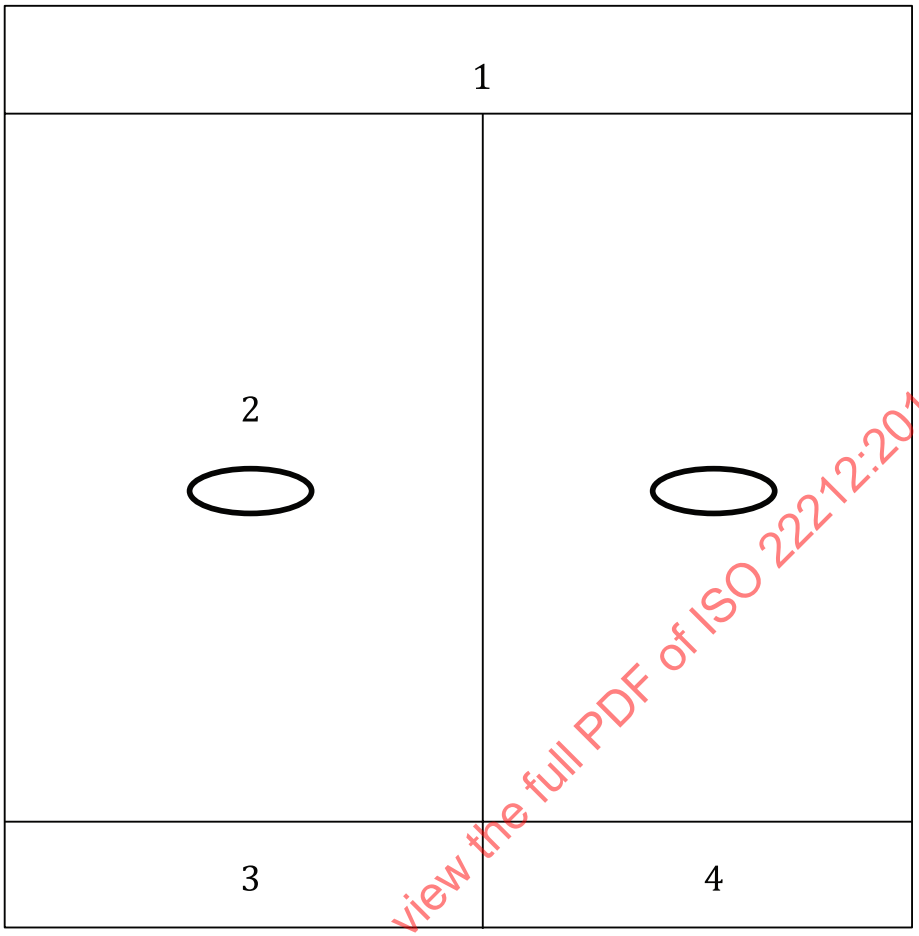
The method specified in Annex C applies.

D.2 Preparation of standard solution

Dissolve *p*-hydroxybenzyl alcohol in ethanol to prepare the standard solution of 1 mg / ml.

D.3 TLC identification

Apply 5 µl of the standard solution and 10 µl of test solution on the same TLC plate (silica gel) previously dried at 110 °C for 15 min in the oven. Develop with a solution of a mixture of petroleum ether (60 °C approximately 90 °C) and ethyl acetate with a volume fraction of 1:1 to a distance of about 10 cm below 10 °C. Take the plate out and dry in air. Spray 10 % phosphomolybdic acid-ethanol solution over the TLC plate and heat at 105 °C until the colour looks clear. Identify the *p*-hydroxybenzyl alcohol spot of test solution by comparing the position and colour with those of the standard solution. A typical TLC chromatogram is shown in Figure D.1. A red-purple spot appears at an *R*f value of about 0,4.



Key

1 top of the plate
2 *p*-hydroxybenzyl alcohol
3 standard solution
4 test solution

Figure D.1 — Schematic diagram of typical TLC chromatogram of *p*-hydroxybenzyl alcohol in *Gastrodia elata* tuber

Annex E
(informative)

Determination of gastrodin and *p*-hydroxybenzyl alcohol contents

E.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through a sieve of 50 mesh or finer. Accurately weigh 2 g of the powder in a 100 ml stopper conical flask. Accurately add 50 ml ethanol. Weigh and sonicate (120 W, 40 kHz) the mixture for 30 min. Cool and weigh again. Replenish the loss of solvent with ethanol and mix well. Filter and transfer 10 ml of the successive filtrate to a round-bottomed flask. Evaporate the solvent to dryness and dissolve the residue in the mixture of acetonitrile and water (3:97). Transfer the solution to a 25-ml volumetric flask. Dilute with the mixture of acetonitrile and water (3:97) to volume and mix. Filter through a 0,45 µm membrane filter as the test solution.

E.2 Preparation of reference standards solution

Dissolve reference standards of gastrodin and *p*-hydroxybenzyl alcohol with the mixture of acetonitrile and water (3:97) to make a solution of 50 µg/ml of gastrodin and 25 µg/ml of *p*-hydroxybenzyl alcohol as the reference standards solution.

E.3 Chromatographic system and high-performance liquid chromatography assay

E.3.1 Column

E.3.1.1 Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

E.3.1.2 Size: l = 0,25 m, $Ø$ = 4,6 mm.

E.3.2 **Mobile phase**: mixture of acetonitrile and 0,05 % phosphoric acid (3:97).

E.3.3 **Flow rate:** 1 ml/min.

E.3.4 **Detector**: 220 nm.

E.3.5 **Injection volume**: 5 µl.

E.3.6 **System suitability requirements**: Perform at least five replicate injections, each using 10 µL of gastrodin standard solution. The requirements of the system suitability parameters are as follows: the RSD of the peak area of gastrodin should not be more than 5,0 %; the RSD of the retention time of gastrodin peak should not be more than 2,0 %; the column efficiency determined from gastrodin peak should not be less than 5 000 theoretical plates.