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Traditional Chinese medicine — *Isatis indigotica* root

*Médecine traditionnelle chinoise — Racine d'*Isatis indigotica

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Foreword

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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).

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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 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

Isatis indigotica root, dried root of *Isatis indigotica* Fort. (Fam. Brassicaceae), is one of the most commonly used herbs in traditional Chinese medicine (TCM). It has a long history of use in East Asian countries to remove heat and toxins, cool the blood and clear the throat.

Clinically, *Isatis indigotica* root is recognized as an important traditional Chinese herb for the prevention and treatment of colds and malignant infectious diseases, especially SARS and H1N1. At present, *Isatis indigotica* root and its processed products occupy a huge share of the international market. However, many problems, such as different quality requirements among different countries and regions, adulteration with Baphicacanthis root derived from the dried root and rhizome of *Baphicacanthus cusia* (Nees) Bremek (Fam. Acanthaceae), and different packaging, transportation and storage conditions, can affect the quality of *Isatis indigotica* root.

Therefore, the establishment of an international standard is necessary to establish the quality requirements of *Isatis indigotica* root to support its clinical effectiveness and safety. This document consists of a morphology observation of macroscopic characteristics, phytochemical indexes, and standardized physical and chemical tests (moisture, total ash and acid-insoluble ash). Glucosinolates with multi-bioactivities are specific compositions of the plant in the Brassicaceae family. Among them, (R,S)-goitrin with high specificity reflects bioactivities relevant to the effects of *Isatis indigotica* root. Using (R,S)-goitrin as the marker, TLC identification and HPLC assay methods are established in this document.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.2, 5.3, 5.4 and 5.5 in their national standards. Examples of national and regional values are given in Annex D.

Traditional Chinese medicine — *Isatis indigotica* root

1 Scope

This document specifies the minimum requirements and test methods for *Isatis indigotica* root derived from the plant *Isatis indigotica* Fort.

It is applicable to *Isatis indigotica* roots that are sold as Chinese material 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*

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

CODEX STAN 229, *Analysis of pesticide residues: Recommended methods*

World Health Organization, *Quality control methods for herbal materials: General advice on sampling*

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
root
dried root of *Isatis indigotica* Fort. (Fam. Brassicaceae)

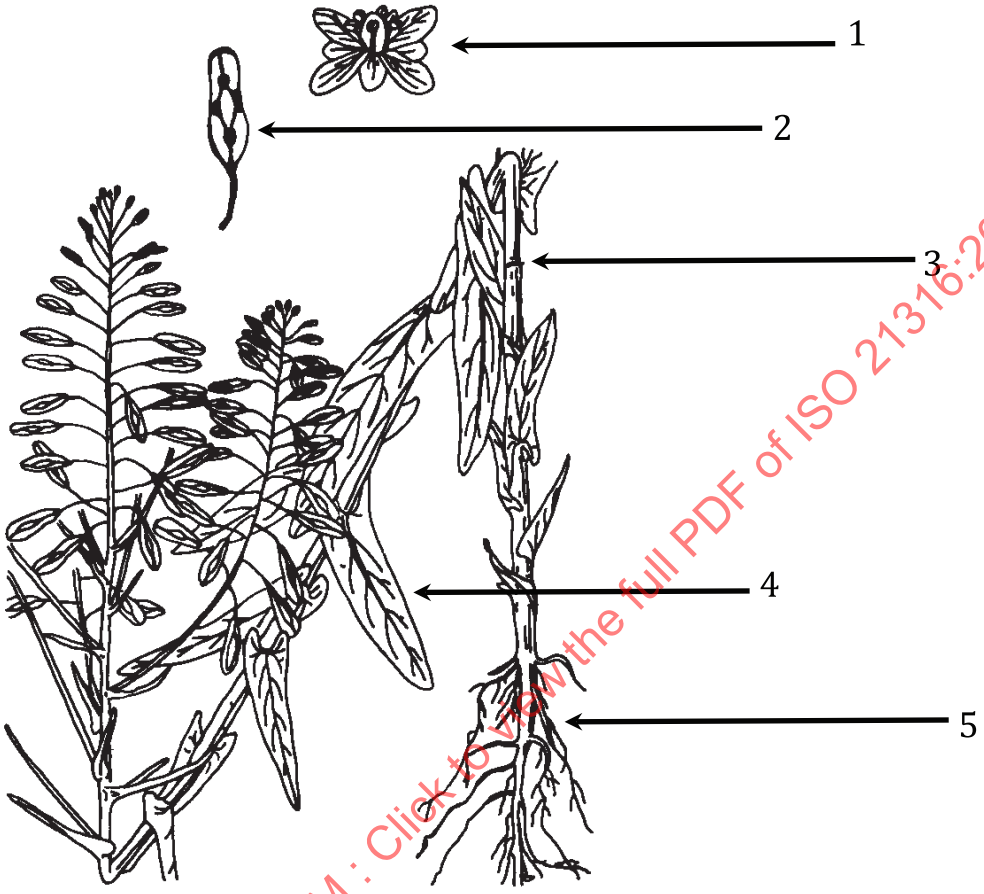
3.2
batch
group of samples collected from the same particular place at the same time, no more than 5 000 kg

3.3
final sample
sample for the test required in this document

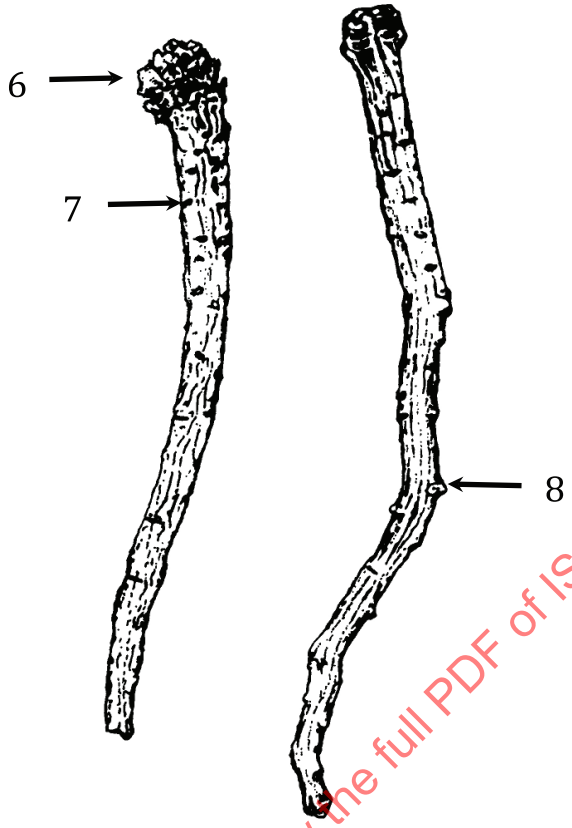
Note 1 to entry: Final samples may be packed in different materials meeting conditions for specific tests (e.g. moisture or total ash).

4 Descriptions

Isatis indigotica root is the dried root of *Isatis indigotica* Fort in the family of Brassicaceae as shown in Figure 1.



a) Plant of *Isatis indigotica* Fort



b) Dried root

Key

1	flower	5	root
2	fruit	6	stem residue
3	stem	7	lenticel
4	leaf	8	rootlet scars

Figure 1 — Structure of *Isatis indigotica* Fort (a) and *Isatis indigotica* root (b)

5 Requirements

5.1 Morphological features

5.1.1 Appearance

The root is cylindrical and slightly twisted, with longitudinal wrinkles, transverse lenticels (7) and rootlets or rootlet scars (8) as shown in Figure 1 b).

5.1.2 Colour

The external surface is pale grayish-yellow to brownish-yellow.

5.1.3 Dimension

The root is 10 cm to 20 cm in length measured from the base to the end of the root and 0,5 cm to 2 cm in diameter measured at the base of the root (1 cm from the position of stem residues).

5.1.4 Stem residue

The base slightly expanded, with dark green or dark brown threaded stem residue (6) as shown in Figure 1 b).

5.1.5 Texture

The texture is compact or slightly soft.

5.1.6 Fracture

The fracture is yellowish-white or yellowish-brown in cortex and yellow or brown in xylem.

5.1.7 Odour

The odour is slight, the taste is at first slightly sweet and then bitter and astringent.

5.2 Moisture

The moisture content in percentage mass should not be more than 15,0 %.

5.3 Total ash

The total ash content in percentage mass should not be more than 9,0 %.

5.4 Acid-insoluble ash

The total 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 25,0 %.

5.6 Identification of maker compound

The identification of marker compound, such as (R,S)-goitrin with thin-layer chromatogram (TLC), shall present spots or brands obtained from the test and reference solutions in the same position with the same colour.

5.7 Content of marker compound

The contents of marker compound, such as (R,S)-goitrin, shall be determined.

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, such as Benzex, DDT and quintozene, shall be determined.

6 Sampling

Sampling of *Isatis indigotica* 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 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 root per batch (kg)	Minimum mass of the final sample (g)		
	For macroscopic identification	For determination of marker compound	For other analyses
1 000	500	250	250
NOTE 1 The requirements are based on samples collected from different production regions of *Isatis indigotica* root.			
NOTE 2 Other analyses include the determination of moisture content, total ash, acid-insoluble content, water-and ethanol-soluble extractives, heavy metals and pesticide residues.			

7 Test methods

7.1 Macroscopic identification

Samples not less than 500 g are taken from each batch randomly. These samples are examined by the naked eye, 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-extractives content

The testing method is as described in Annex A.

7.6 Thin-layer chromatogram (TLC) identification

The testing method is as described in Annex B.

7.7 Determination of marker compound content

The testing method is as described in Annex C.

7.8 Determination of heavy metals contents

The testing method specified in ISO 18664 applies.

7.9 Determination of pesticide residues contents

The testing method specified in CAC/MRL01 and CODEX STAN 229 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 may 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 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 product drug shall be sealed and stored in a dry, shady and cool place.

The *Isatis indigotica* root 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 applies. 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/quantity;

d) contact information;

e) name of raw materials;

f) warning statements, if any;

g) expiry date;

h) storage method;

i) batch/lot number;

j) miscellaneous.

Annex A
(informative)

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 mass. Weigh approximately 4 g of the dried powder into a 250-ml stopper conical flask. Accurately add 100 ml ethyl alcohol absolute (ethanol) and weigh.

b) Heat the mixture of the powder and ethanol 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 the 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 extracts rapidly and accurately.

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

$$m_{ese} = \left(m_1 - m_0\right) \times 4 / m_S \times 100\ \% \qquad \text{(A.1)}$$

where

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 *Isatis indigotica* root to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 1 g of the powder, add 20 ml of 80 % methanol-water (v/v), sonicated for 30 min and filter. Evaporate the filtrate to dryness then dissolve the residue with 1 ml of methanol as the sample solution.

b) Weigh 1 g of *Isatis indigotica* root reference drug powder and treat it in the same manner as in a) as the reference drug solution.

c) Dissolve a quantity of (R,S)-goitrin CRS in methanol to produce a solution containing 0,5 mg per ml as the reference solution.

B.2 Developing solvent system

Prepare a mixture of petroleum ether (60 °C~90 °C) and ethyl acetate in the volume ratio of 1:1 as the mobile phase.

B.3 Procedure

Apply 10 µl each of the reference standard solution, reference drug solution and test solution on the same TLC plate (silica gel GF_{254}) previously dried at 110 °C for 15 min in the oven. Develop the plate in the above mobile phase, then take the plate out and dry in air. Examine the plate under ultraviolet light at 254 nm. Identify the spots of (R,S)-goitrin and other spots of the test solution by comparing the positions and colours with these of the reference standard solution and reference drug solution. Typical reference TLC chromatograms are shown in Figure B.1.

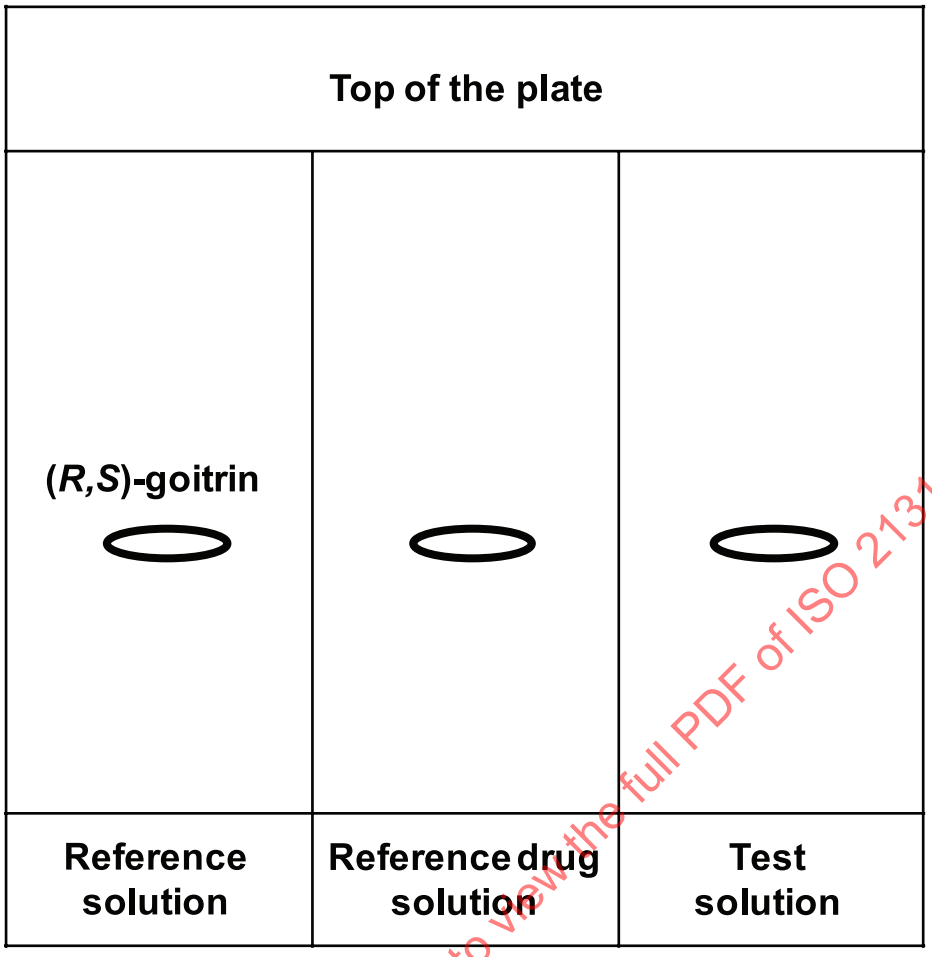


Figure B.1 — Schematic diagram of typical TLC chromatogram of *Isatis indigotica* root

Annex C
(informative)

Determination of (R,S)-goitrin content

C.1 Preparation of reference standard solution

Dissolve a quantity of (R,S)-goitrin CRS with methanol to make a solution containing 40 µg per ml as the reference standard solution.

C.2 Preparation of test solution

Weigh 250 g of *Isatis indigotica* root to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 1 g of the powder in a 100 ml round-bottom flask. Accurately add 50 ml of water. Weigh and heat it under reflux to slightly boil on a water bath at 80 °C for 2 h. Cool and weigh again. Replenish the loss of solvent with water and mix well, filter and use the successive filtrate. Filter through a 0,45 µm membrane filter as the test solution.

C.3 Chromatographic system

C.3.1 Column

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

C.3.1.2 Size: *l* = 250 mm, Ø = 4,6 mm.

C.3.2 Mobile phase

C.3.2.1 Mobile phase A: 0,02 % (v/v) phosphoric acid in water.

C.3.2.2 Mobile phase B: methanol.

C.3.2.3 Isocratic elution: a mixture of mobile phases A and B (93:7).

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

C.3.4 Detector: 245 nm.

C.3.5 Column temperature: 30 °C.

C.3.6 Injection volume: 10 µl.