



**International
Standard**

ISO 19025

**Traditional Chinese medicine —
Glycyrrhiza uralensis, *Glycyrrhiza
inflata*, and *Glycyrrhiza glabra* root
and rhizome**

*Médecine traditionnelle chinoise — Rhizome et racine de
Glycyrrhiza uralensis, Glycyrrhiza inflata et Glycyrrhiza glabra*

**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 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

Glycyrrhizae root and rhizoma, also known as Guolao, Lingtong, Sweet grass and Lolium, is the dried root and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat or *Glycyrrhiza glabra* L. It is included in the Pharmacopoeia of the People's Republic of China, the Japanese Pharmacopoeia, the United States Pharmacopoeia, Korea Pharmacopoeia and European Pharmacopoeia. It has been widely used as an important medicinal herb around the world, such as in China, Japan, Turkey, Greece and Egypt. It's one of the oldest Chinese medicinal herbs which has high levels of bioactive phytochemicals. In ancient times, it was commonly used to treat oral ulcers, indigestion, and hemorrhoids. Besides, this plant has multiple pharmacological properties, including anti-inflammatory, antiviral, antimicrobial, antioxidative, anticancer, and antidiabetic effects.

Licorice products are mainly derived from cultivated *Glycyrrhizae* root and rhizoma plants from China or other Asian countries. For licorice, different extracts have different effects and, in some cases, do not have any effects. Therefore, consistent quality of *Glycyrrhizae* root and rhizoma cultivated is a prerequisite to ensure safety and efficacy in its preparations and production. However, wide variations in bioactive components contents of *Glycyrrhizae* root and rhizoma cultivated were observed, which are influenced by various factors, such as genetic differences, environmental factors, and agronomic practices. In addition, different countries, such as China, Japan and the United States, have different quality standards for the quality markers or other components about *Glycyrrhizae* root and rhizoma.

The establishment of an international standard for *Glycyrrhizae* root and rhizoma 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 [5.4](#), [5.5](#), and [5.6](#) in their national standards. Examples of national and regional values are given in [Annex C](#).

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Traditional Chinese medicine — *Glycyrrhiza uralensis*, *Glycyrrhiza inflata*, and *Glycyrrhiza glabra* root and rhizome

1 Scope

This document specifies the quality and safety requirements for *Glycyrrhiza* root and rhizome that is derived from the root and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat or *Glycyrrhiza glabra* L. (Fam. Fabaceae).

This document applies to *Glycyrrhiza* root and rhizome that is sold and used as natural medicines 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 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 22217:2020, *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 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/>

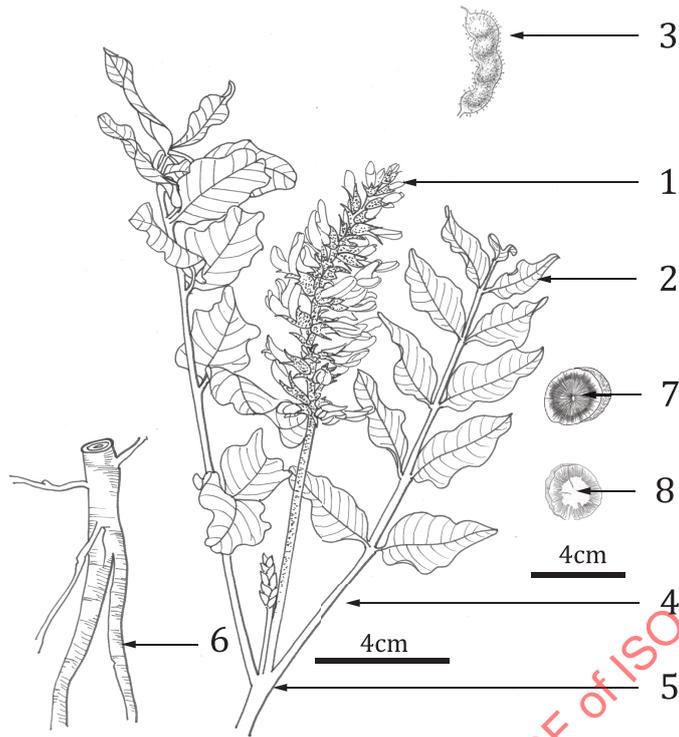
3.1

***Glycyrrhiza* root and rhizome**

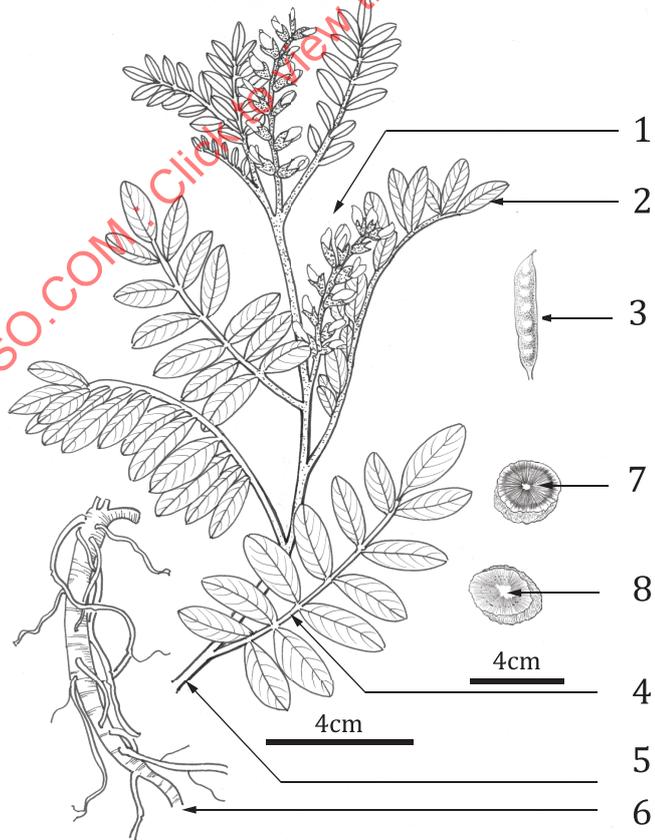
dried root and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat or *Glycyrrhiza glabra* L. (Fam. Fabaceae) and their hybrids

4 Descriptions

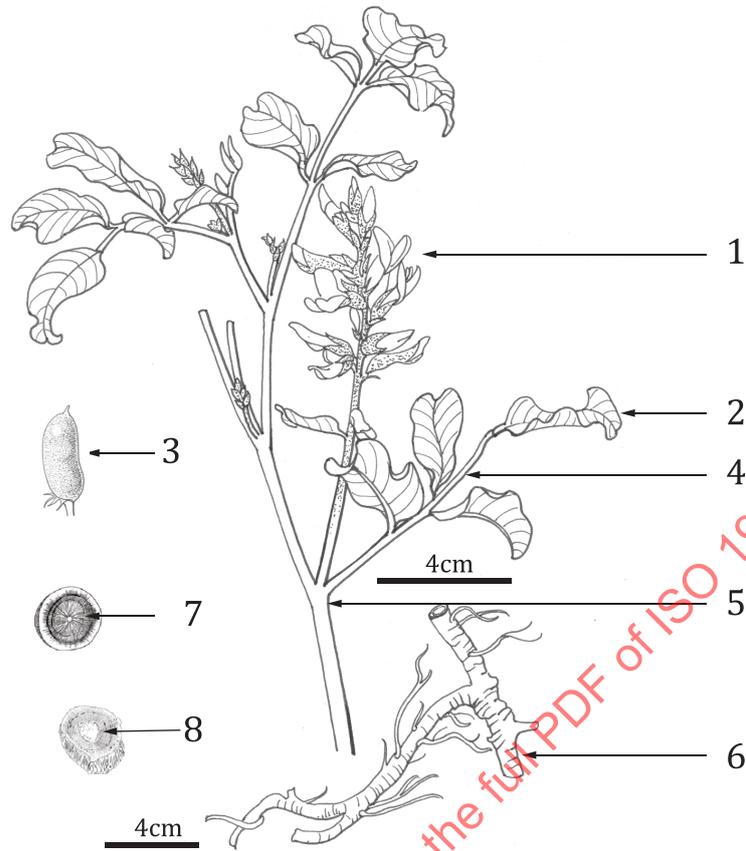
Glycyrrhiza root and rhizome is the dried root or rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., *Glycyrrhiza glabra* L. (Fam. Fabaceae) in the family of Leguminosae shown in [Figure 1](#).



a) Plant of *Glycyrrhiza uralensis* Fisch.



b) Plant of *Glycyrrhiza glabra* L.



c) Plant of *Glycyrrhiza inflata* Bat

Key

- 1 inflorescence
- 2 leaf
- 3 pod
- 4 petiolate
- 5 stem
- 6 root and rhizome
- 7 xylem, cambium and phloem
- 8 pith

Figure 1 — Structure of *Glycyrrhiza* root and rhizome

5 Requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling.

- *Glycyrrhiza* root and rhizome shall be clean and free from foreign matter.
- 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 Morphological characteristics

The morphological characteristics of *Glycyrrhiza* root and rhizome are given in [Table 1](#).

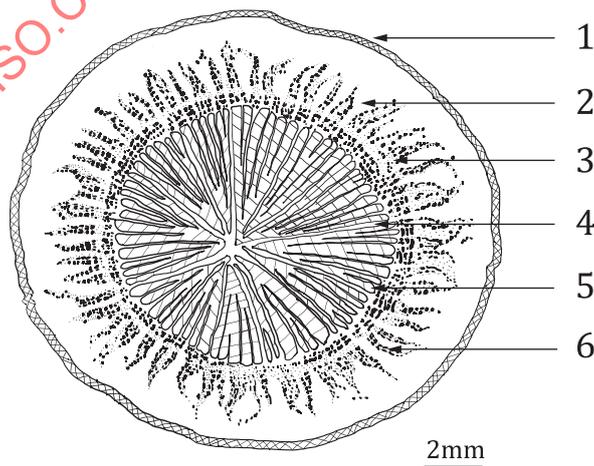
Table 1 — Morphological characteristics of *Glycyrrhiza* root and rhizome

Licorice species	Morphological character
<i>Glycyrrhiza uralensis</i>	The root is cylindrical, 25 cm to 100 cm long, 0,6 cm to 3,5 cm in diameter. The outer bark can be loose or tight. The surface is reddish-brown or greyish-brown, obviously longitudinally wrinkled, furrowed, lenticel-like protruded, and with sparse rootlet scars. The texture is compact, fracture slightly fibrous, yellowish-white, starchy, cambium ring distinct, rays radiate, some with clefts. Rhizomes are cylindrical, externally with bud scars, pith present in the centre of fracture. The odour is slight; the taste is sweet.
<i>Glycyrrhiza glabra</i>	The texture of root and rhizomes is relatively compact, some branched; the outer bark is not rough, mostly greyish-brown; lenticels are small and indistinct.
<i>Glycyrrhiza inflata</i>	Root and rhizomes are woody and stout, some branched; the outer bark is rough, mostly greyish-brown. The texture is compact, with abundant lignified fibres, and less starchy than <i>Glycyrrhiza uralensis</i> . Rhizomes have more and large adventitious buds comparative to <i>Glycyrrhiza uralensis</i> .

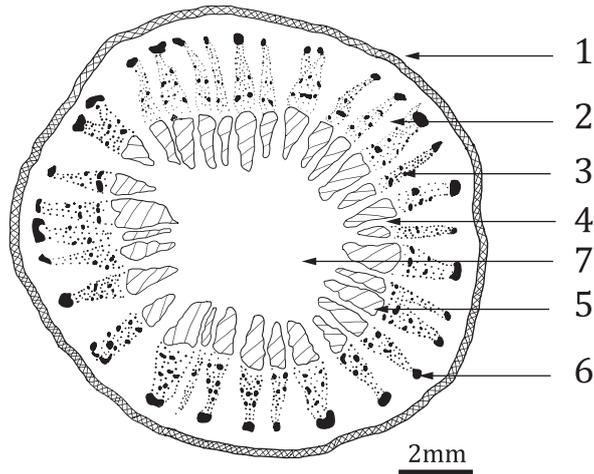
5.3 Microscopic characteristics

The transverse section is as shown in [Figure 2](#).

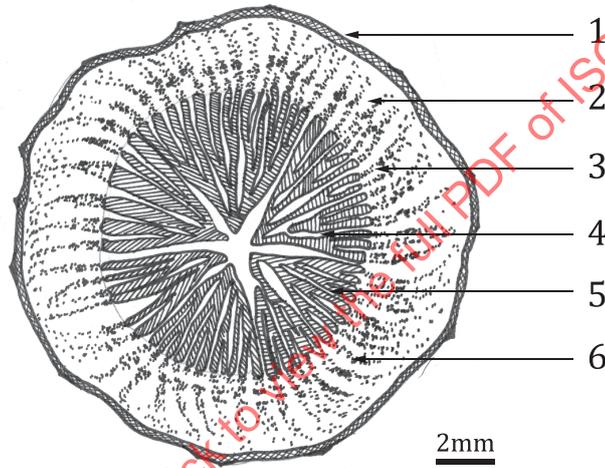
- The transverse section reveals several yellow-brown cork layers, and a layer of phelloderm that is 1 to 3 cells thick.
- The cortex exhibits medullary rays, and obliterated sieve portions radiate alternately.
- The phloem exhibits groups of phloem fibres, which are surrounded by crystal cells, with thick but incompletely lignified walls.
- The vessels are accompanied by xylem fibres, which are surrounded by crystal cells, and by xylem parenchyma cells.
- The parenchyma cells contain starch grains and often contain single crystals of calcium oxalate.
- The root doesn't have a pith at the centre, but the rhizome does.



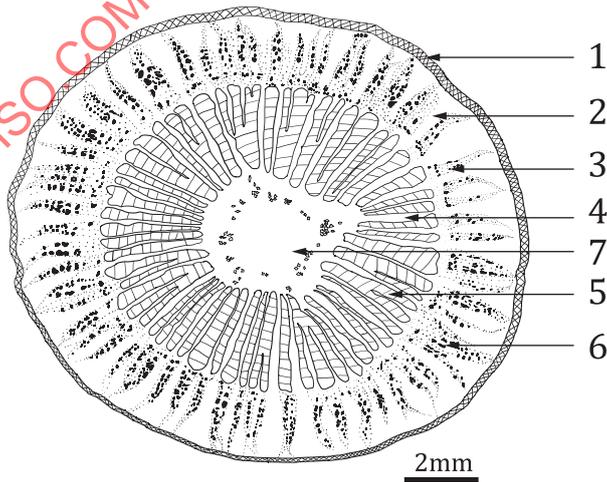
a) *Glycyrrhiza uralensis* root



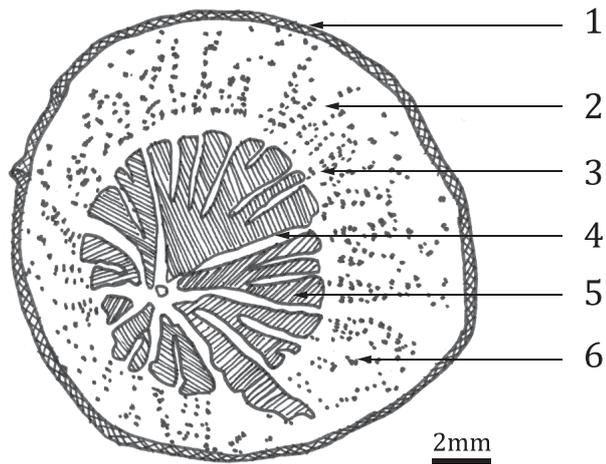
b) *Glycyrrhiza uralensis* rhizome



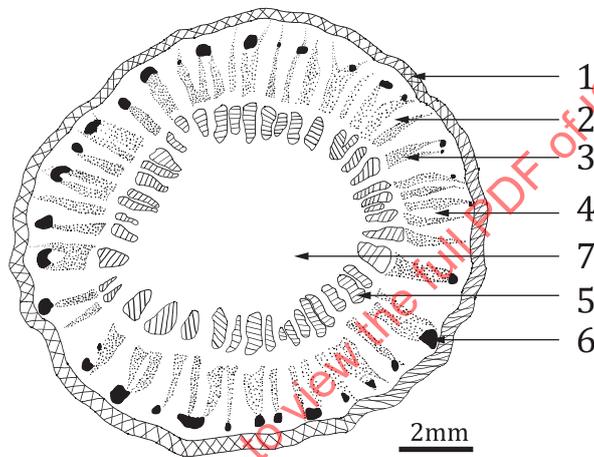
c) *Glycyrrhiza inflata* root



d) *Glycyrrhiza inflata* rhizome



e) *Glycyrrhiza glabra* root



f) *Glycyrrhiza glabra* rhizome

Key

- 1 phellem
- 2 phloem ray
- 3 phloem
- 4 xylem ray
- 5 xylem
- 6 phloem fibre
- 7 pith

Figure 2 — Transverse section of *Glycyrrhiza* root and rhizome

5.4 Moisture

The content of moisture as a mass fraction should not be more than 12,0 %.

5.5 Total ash

The content of total ash as a mass fraction should not be more than 7,0 %.

5.6 Acid-insoluble ash

The content of acid-insoluble ash as a mass fraction should not be more than 2,0 %.

5.7 Thin-layer chromatogram identification

The thin-layer chromatogram (TLC) of *Glycyrrhiza* root and rhizome shall present the spots and bands with the same colour and position corresponding to those of reference solutions.

5.8 Marker compounds

The content of marker compounds, such as glycyrrhizic acid and liquiritin, as mass fractions should be determined.

5.9 Heavy metals

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

5.10 Pesticide residues

The content of pesticide residues, such as organo-chlorine pesticide, as mass fractions shall be determined.

6 Sampling

Sampling shall be carried out in accordance with the method described in ISO 23723:2021, Clause 8.

7 Test methods

7.1 Macroscopic identification

Test samples of not less than 250 g shall be observed with the naked eye, smelled and tasted. The length and diameter of the root shall be measured.

7.2 Determination of moisture content

The test method specified in ISO 23723:2021, 7.2.1 shall apply.

7.3 Determination of total ash content

The test method specified in ISO 23723:2021, 7.2.3 shall apply.

7.4 Determination of acid-insoluble ash

The test method specified in ISO 23723:2021, 7.2.3 shall apply.

7.5 Thin-layer chromatogram identification

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

7.6 Determination of marker compounds

See [Annex B](#) for additional information on determination of marker compounds by HPLC-UV.

7.7 Determination of heavy metals content

The test method specified in ISO 18664 shall apply.

7.8 Determination of pesticide residues content

The test method specified in ISO 22258 shall apply.

8 Test report

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

- all information necessary for the complete identification of the sample;
- the sampling method used;
- the test method used, with reference to this document;
- the test result(s) obtained;
- 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);
- any unusual features (anomalies) observed during the test;
- 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 22217:2020, 5.2 shall apply.

The products should be ventilated and protected from moisture, pollution and moth during storage.

10 Marking and labelling

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

- the product name and Latin scientific name of the original plant;
- all quality features indicated in [Clause 5](#) determined in accordance with the methods specified in [Clause 7](#);
- gross mass and net mass of the package;
- the country and province or state of origin of the product, as well as the name, trademark or logo of the producer and supplier;
- the production date, batch number and expiry date of the product;
- the storage method;
- expiry date.

Annex A
(informative)

Thin-layer chromatogram identification test

A.1 Standard solution

Dissolve a quantity of chemical reference standard (CRS) of glycyrrhizic acid in 70 % alcohol to produce a solution containing 5 mg per ml as the reference solution.

A.2 Sample solution

Dissolve 2 g of pulverized *Glycyrrhiza* root and rhizome in 10 ml of a mixture of alcohol and water (7:3). Heat the solution by shaking on a water bath for 5 min, cool, and filter.

A.3 Chromatographic system

Adsorbent: 0,25 mm layer of chromatographic silica gel mixture (TLC plates).

Application volume: 2 µl.

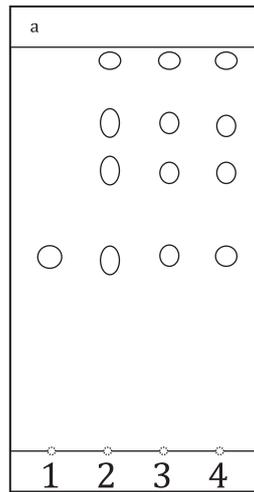
Developing solvent system: Butyl alcohol, glacial acetic acid, and water (7:1:2).

A.4 Analysis

Samples: standard solution and sample solution.

Develop the chromatogram in an unsaturated chamber to a length of 10 cm. Examine the plate under 254 nm light.

Acceptance criteria: The chromatograms show a dark purple zone, among other spots, due to glycyrrhizic acid at an RF value of 0,4. See [Figure A.1](#).



Key

- 1 reference solution of glycyrrhizic acid
- 2 test solution of *Glycyrrhiza uralensis* root and rhizome
- 3 test solution of *Glycyrrhiza inflata* root and rhizome
- 4 test solution of *Glycyrrhiza glabra* root and rhizome
- a Top of the plate.

Figure A.1 — TLC chromatograms of *Glycyrrhiza* root and rhizome

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Annex B (informative)

Determination of marker compounds by HPLC-UV

B.1 Preparation of test solution

Weigh 0,2 g of the *Glycyrrhiza* root and rhizome powder (through an 50-mesh sieve) to a stopper conical flask, add 100 ml of 70 % ethanol, tightly seal the flask with the stopper and weigh. Ultrasonicate (power 250 W; frequency 40 kHz) for 30 min, cool and weigh again, replenish the loss of solvent with 70 % ethanol, mix well, filter and use the successive filtrate as the test solution.

B.2 Preparation of reference solution

Dissolve a quantity of ammonium glycyrrhizate CRS in 70 % ethanol to produce a solution containing 0,2 mg per ml (the mass of glycyrrhizic acid equal to the mass of ammonium glycyrrhizate divided by 1,027), respectively.

If liquiritin is also quantified, dissolve a quantity of liquiritin CRS in 70 % ethanol to produce a second solution containing 20 µg of liquiritin.

B.3 Chromatographic conditions

- Stationary phase: octadecylsilane bonded silica gel.
- Column size: 5 µm, 4,6 mm × 150 mm.
- Mobile phase: acetonitrile (A) and 0,05 % solution of phosphoric acid (B). The gradient elute conditions are shown in [Table B.1](#).
- Flow rate: 1 ml/min.
- Injection volume: 10 µl.
- Detector: a spectrophotometer set at 237 nm (liquiritin) and 254 nm (glycyrrhizic acid).

Table B.1 — gradient elute conditions

Time (min)	Mobile phase A (volume fraction in %)	Mobile phase B (volume fraction in %)
0 to 8	19	81
8 to 35	19 → 50	81 → 50
35 to 36	50 → 100	50 → 0
36 to 40	100 → 19	0 → 81

B.4 Determination

Inject 10 µl of each of the reference solution and the test solutions, respectively, into the column, and calculate the content. The HPLC chromatograms of *Glycyrrhiza* root and rhizome is shown in [Figure B.1](#).