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Traditional Chinese medicine — *Polygala tenuifolia* and *Polygala sibirica* root

Médecine traditionnelle chinoise — Racine de Polygala tenuifolia *et* Polygala sibirica

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Foreword

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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

Polygala root is the dried root of *Polygala tenuifolia* Willd. or *Polygala sibirica* L. (Polygalaceae). It has the functions of soothing the nerves, promoting heart and kidney health, removing phlegm, and reducing swelling. It is used for insomnia, dreaminess, trance, uncomfortable expectoration, sore swelling, breast swelling and pain. The medicinal materials, decoction pieces and extracts of Polygala root are widely sold in China, Japan, Republic of Korea, Southeast Asia, North America and other regions. Polygala root has been included in Chinese Pharmacopoeia, Japanese Pharmacopoeia, Korean Pharmacopoeia and European Pharmacopoeia, but their requirements and test methods are different. Besides, there are traditional Chinese medicine (TCM) such as *Liriope spicata* root on the market, which is usually confused with Polygala root; and the aflatoxins in Polygala root may exceed the maximum residue limit if stored improperly. In addition, the grade of medicinal materials is not linked to the intrinsic quality. These problems can affect the international trade of Polygala root. Therefore, it is necessary to establish an International Standard to bring benefits to the consumers, enterprises and companies involved in the processing, management and trade of *Polygala tenuifolia* and *Polygala sibirica* root, and ensure its quality and safety.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.5 in their national standards. Examples of national and regional values are given in Annex C.

Traditional Chinese medicine — *Polygala tenuifolia* and *Polygala sibirica* root

1 Scope

This document specifies the minimum requirements and test methods for *Polygala tenuifolia* and *Polygala sibirica* root.

It applies to *Polygala tenuifolia* and *Polygala sibirica* root that are sold and used as natural medicines in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces.

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 15378, *Primary packaging materials for medicinal products — Particular requirements for the application of ISO 9001:2015, with reference to good manufacturing practice (GMP)*

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

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*

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
root
part of a higher plant growing under the stem in the soil

3.2
***Polygala* root**
root (3.1) of *Polygala tenuifolia* Willd. or *Polygala sibirica* L.

3.3
powder
fine particles of matter in a solid state

3.4
total ash
residue obtained after incineration at 525 °C ± 25 °C

[SOURCE: ISO 22584:2019, 3.3]

3.5
marker compound
chemical constituent within a medicinal herb that can be used to verify its quality

Note 1 to entry: Usually described as active ingredients or chemicals that confirm the correct botanical identity of the starting material.

[SOURCE: ISO 22586:2022, 3.2, modified — Note 2 to entry has been removed.]

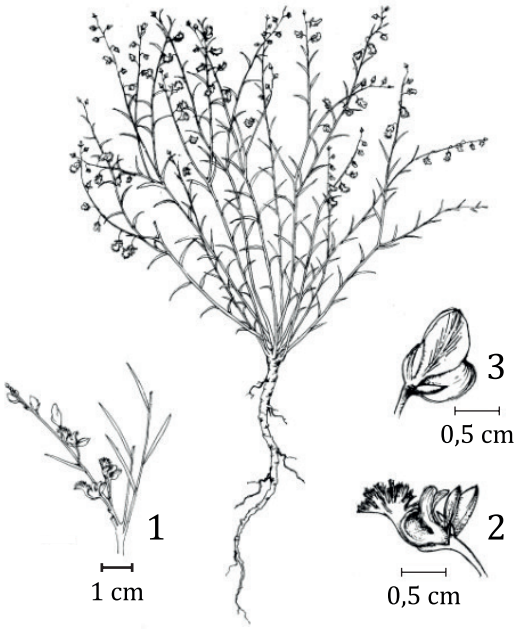
3.6
reference medicine
authentic medicine from the *Polygala root* (3.2), used for reference in TLC analyses of the sample

3.7
sample batch
samples collected from the same particular place at the same time

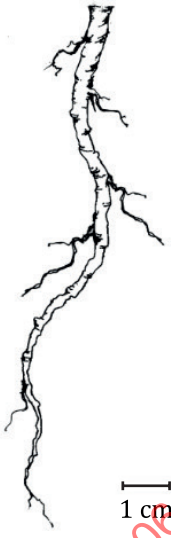
[SOURCE: ISO 21317:2019, 3.5]

4 Description

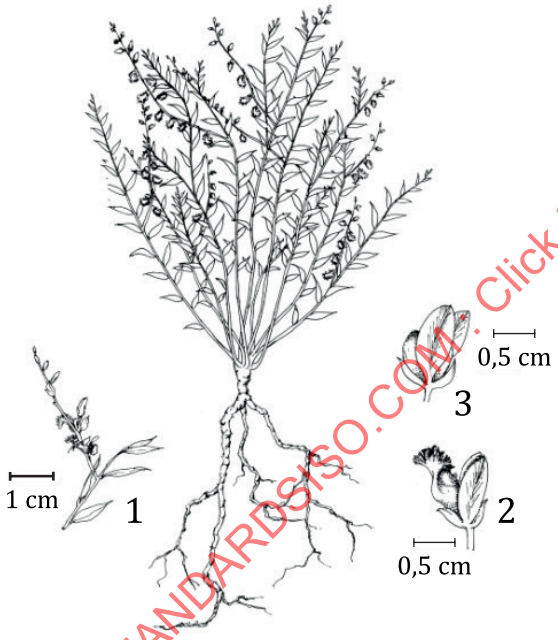
Polygala root is the dried root of *Polygala tenuifolia* Willd. and *Polygala sibirica* L. in the family of *Polygalaceae* as shown in Figure 1.



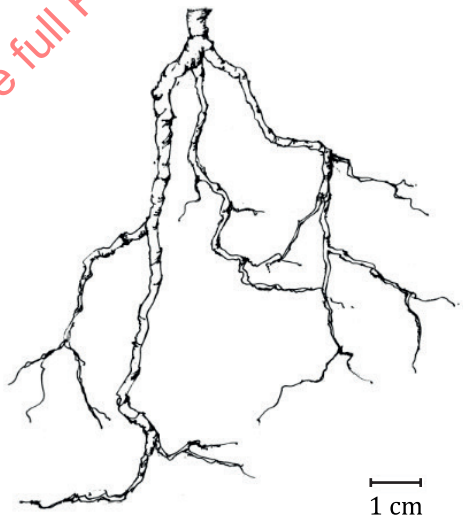
a) Plant of *Polygala tenuifolia* Willd.



b) *Polygala tenuifolia* root



c) Plant of *Polygala sibirica* L.



d) *Polygala sibirica* root

Key

1 flowering branch
2 flower
3 fruit

Figure 1 — Morphological structure of *Polygala tenuifolia* and *Polygala sibirica* root

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling.

a) *Polygala tenuifolia* and *Polygala sibirica* root materials shall be dry.

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

5.2 Macroscopic characteristics

Polygala tenuifolia root is cylindrical, long, thin and curved. The main root is 2 cm to 30 cm in length and 0,2 cm to 1 cm in diameter. The external surface is pale greyish yellow to greyish brown with a relatively dense and deeply dented transverse wrinkles, longitudinal wrinkles and open gaps. Older roots have relatively dense transverse wrinkles, even more deeply dented, slightly knotted. The texture is hard, fragile and easy to cut. The cut surface has a yellowish-brown cortex and yellowish-white xylem. The cortex and xylem are separate and easily detached, sometimes with the core already removed.

Different from *Polygala tenuifolia* root, *Polygala sibirica* root is forked more.

Polygala root has a faint odour and a slightly acrid taste.

5.3 Microscopic characteristics

5.3.1 Transverse section

The transverse section of *Polygala tenuifolia* root reveals a cork layer consisting of about 10 rows of cork cells. The cortex is narrow and the phloem is relatively wide with open gaps throughout. The cambium forms a ring. Those from which the core has not been removed have a xylem. Several vessels form groups and are scattered, surrounded by lignified xylem fibre bundles. The xylem rays consist of 1 to 3 rows of cells, most parenchyma cells contain fatty oil drops, sometimes containing calcium oxalate druses or solitary crystals.

For *Polygala sibirica* root, the outside of xylem is flat, without awn tooth structure.

5.3.2 Powder

The *P. tenuifolia* powder shows the following diagnostic characters:

a) fragments of lignified tissue made up of numerous pitted tracheids and slightly larger, reticulate, pitted or bordered-pitted vessels;

b) yellowish parenchyma cells containing oil droplets;

c) fragments of cork, sometimes accompanied by phelloderm and parenchyma, some cells of which contain oil droplets;

d) numerous isolated oil droplets;

e) *P. tenuifolia* powder also containing calcium oxalate cluster crystals, isolated or included in parenchymatous cells;

f) long, fine, thick-walled lignified fibres, most often fragmented, in clusters or associated with vessels.

For *Polygala sibirica* root powder, there are abundant round granules in the inner layer of thrombus, but no oil droplets.

5.4 Moisture

The mass fraction of moisture should be determined.

5.5 Total ash

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

5.6 Thin layer chromatogram

The chromatogram of sample solution should exhibit specific spots to *Polygala tenuifolia* or *Polygala sibirica* root.

5.7 Ethanol-soluble extractives

The mass fractions of ethanol-soluble extracts should be determined.

5.8 Content of marker compound(s)

The mass fraction(s) of marker compound(s) such as tenuifolin should be determined.

5.9 Mycotoxins

The mass fractions of aflatoxins (B_1, B_2, G_1, G_2) should be determined.

5.10 Heavy metals

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

5.11 Pesticide residues

The mass fractions of pesticide residues such as benzene hexachloride (BHC), dichlorodiphenyltrichloroethane (DDT) and pentachloronitrobenzene (PCNB) should be determined.

5.12 Sulfur dioxide residues

The mass fractions of sulfur dioxide residues should be determined.

6 Sampling

Sampling of *Polygala tenuifolia* and *Polygala sibirica* root shall be carried out in accordance with the method specified in ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples of not less than 500 g are taken from each sample batch randomly and observed with the naked eye and smelled.

7.2 Thin-layer chromatographic identification

See Annex A for additional information.

7.3 Determination of moisture

The test method specified in ISO 23723 shall apply.

7.4 Determination of total ash and acid-insoluble ash

The test method specified in ISO 23723 shall apply.

7.5 Determination of ethanol-soluble extractives

The test method specified in ISO 23723 shall apply.

7.6 Determination of marker compound(s)

See Annex B for additional information.

7.7 Determination of mycotoxins

The test method specified in ISO 22283 shall apply.

7.8 Determination of heavy metals

The test method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues

The test methods specified in ISO 22258 shall apply.

7.10 Determination of sulfur dioxide residues

The test method specified in ISO 22590 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 materials shall be as specified in ISO 15378. The packaging and transportation 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 storage requirements specified in ISO 22217 shall apply.

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) product name;

b) category of the product in the marketed country or region;

c) net mass or quantity;

d) contact information;

e) name of raw materials;

f) date of production and expiry date of the products;

g) storage method;

h) sample batch or lot number.

Annex A
(informative)

Thin-layer chromatographic identification

A.1 Reagents

A.1.1 Water, which shall be distilled water.

A.1.2 Ethyl acetate, glacial acetic acid, analytical grade.

A.1.3 Developing agent, ethyl acetate : glacial acetic acid : water (55:13:13, volume ratio).

A.1.4 Prefabricated thin layer high-efficiency silica gel F_{254} or equivalent plate, 100 mm × 200 mm high-efficiency silica gel F_{254} plate, activated at 105 °C for 30 min before use.

A.2 Apparatus

A.2.1 Sieve, sieve hole diameter (average): 850 µm ± 29 µm, 24 mesh.

A.2.2 Conical flask, 100 ml.

A.2.3 Analytical balance, weighing accuracy 0,01 mg.

A.2.4 Constant temperature blast oven, variable temperature range 30 °C to 300 °C, temperature control accuracy of ±1 °C.

A.2.5 UV detection lights, 365 nm.

A.3 Sample analysis

A.3.1 Preparation of the test solution

Weigh 0,5 g of the powdered sample, then add 5 ml of 70 % ethanol. Sonicate the mixture for 15 min. Filter and the filtrate is used as the test solution.

A.3.2 Preparation of reference medicine solution

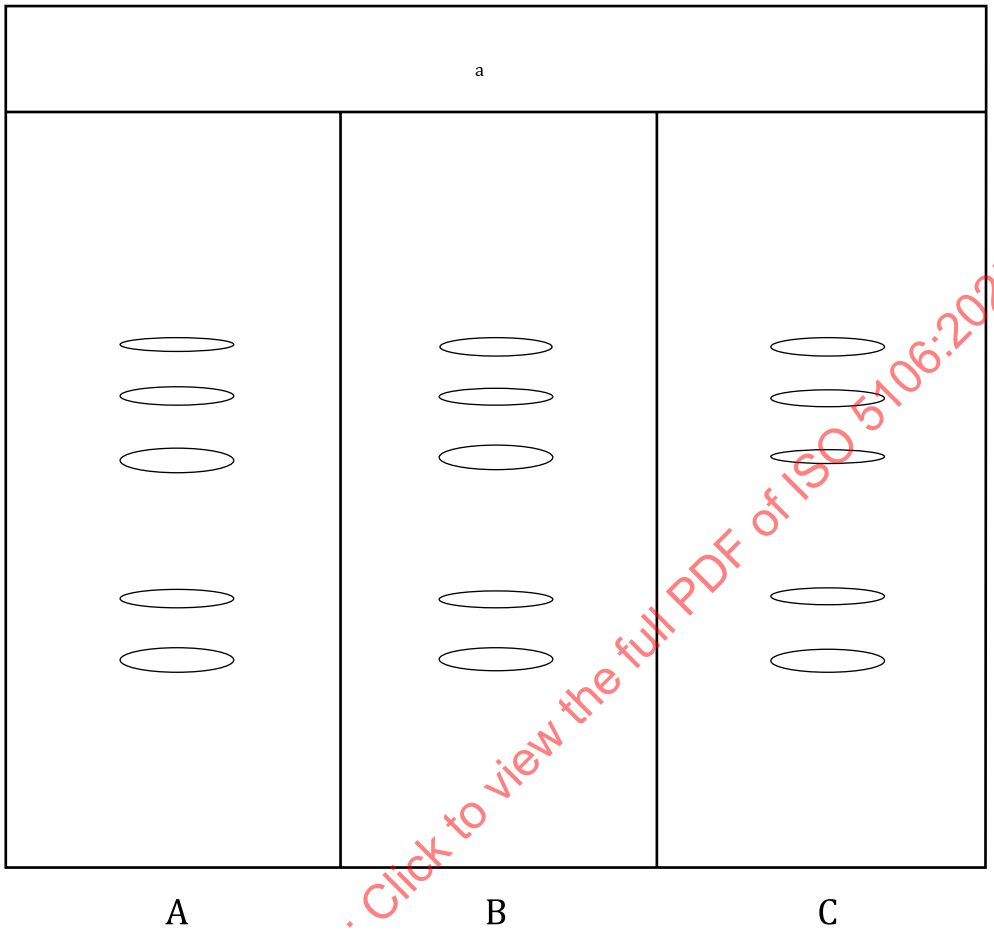
Weigh 0,5 g of the reference medicine, and prepare the reference medicine by the same method as described in A.3.1.

A.3.3 Identification by TLC

Carry out the method by using a HPTLC silica G plate and a freshly prepared developing agent as described in A.1.3. Apply separately the reference medicine solution (2 µl) and the test solution (2 µl) to the plate, develop. Remove the plate from the chamber, mark the solvent front, allow the plate to dry in air for about 1 min and examine in ultraviolet light at 365 nm.

A.4 Record the result

In the same high-efficiency silica gel G plate, the chromatogram of the test solution is corresponding to the chromatogram of the reference medicine, and the same fluorescent spots are displayed. Typical reference TLC chromatograms are shown in Figure A.1.



Key

A reference medicine

B *Polygala tenuifolia* root

C *Polygala sibirica* root

[a] Top of the plate.

Figure A.1 — Schematic diagram of typical TLC chromatogram of *Polygala tenuifolia and Polygala sibirica root*

Annex B
(informative)

Determination of marker compound(s)

B.1 Reagents

B.1.1 **Water**, which shall be distilled water.

B.1.2 **Methanol, N-butanol, phosphoric acid solution**, analytical grade.

B.1.3 **Acetonitrile**, chromatographic grade.

B.1.4 **Tenuifolin reference substance**, purity ≥ 98 % (Figure B.1).

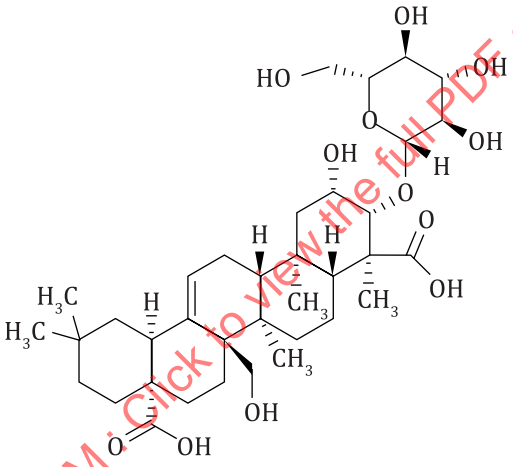


Figure B.1 — Chemical structures of tenuifolin

B.2 Apparatus

B.2.1 **Analytical balance**, weighing accuracy 0,01 mg.

B.2.2 **Ultrasonicator**, power 400 W, frequency 40 kHz.

B.2.3 **HPLC-UV system**, UV (210 nm).

B.3 Preparation of reference standard solution

Dissolve reference substance of tenuifolin in methanol to prepare the reference standard solution of 1,0 mg per ml, store at −20 °C before use.

B.4 Preparation of the test solution

Weigh 1,0 g of the powder. Add 50 ml of 70 % ethanol, weigh, and sonicate for 60 min (with the power 400 W, frequency 40 kHz), cool down, and weigh again. Add 70 % ethanol to make up for lost weight, shake well and

filter. Measure 25 ml of the subsequent filtrate into a round-bottomed flask, steam dry. Add 50 ml of 10 % sodium hydroxide solution, reflux for 2 h, cool down, adjust pH to 4 to 5 with hydrochloric acid, extract 3 times with water-saturated *n*-butanol, 50 ml at each time. Combine the *n*-butanol liquid and evaporate to dryness, dissolve the residue with methanol, transfer to a 25 ml volumetric flask, add methanol to the mark, shake well. Filter through a 0,45-µm nylon filter, take the filtrate as the test solution.

B.5 Chromatographic system and HPLC assay

B.5.1 Column.

B.5.1.1 Stationary phase, octadecylsilane bonded silica gel as analysing column or equivalent.

B.5.1.2 Size, l = 0,25 m, $\varnothing$ = 4,6 mm.

B.5.2 Mobile phase, methanol-0,05 % phosphoric acid solution (70:30, volume ratio).

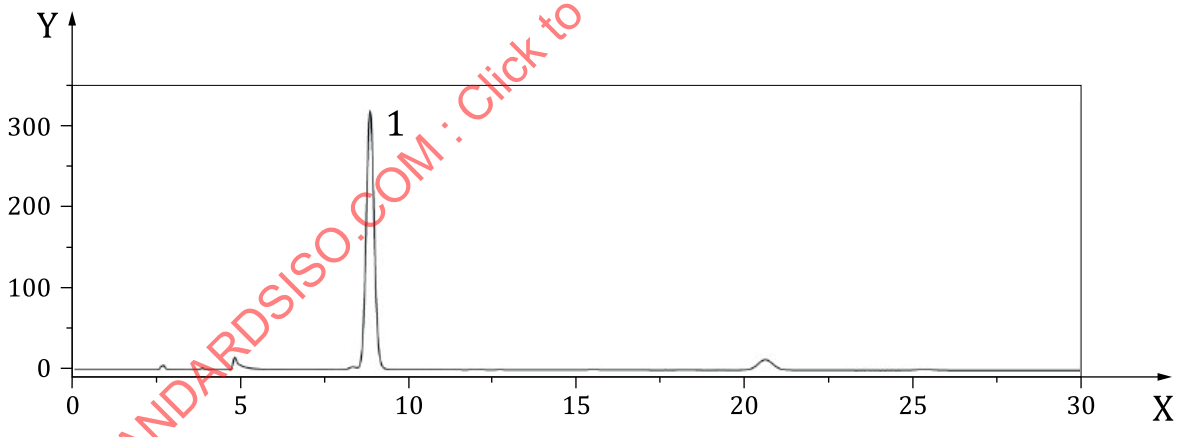
B.5.3 Detector, 210 nm.

B.5.4 Injection volume, 10 µl.

The number of theoretical plates calculated tenuifolin a peak should not be less than 3 000.

B.6 Record the result

Calculate the mass fraction of tenuifolin in the test sample according to the standard curve, and calculate the mass fraction of tenuifolin in the dried medicinal materials according to the water mass fraction. Typical reference HPLC chromatograms are shown in Figure B.2.



a) Chromatogram of reference standard solution