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Traditional Chinese medicine — *Saposhnikovia divaricata* root and rhizome

Médecine traditionnelle chinoise — Racine et rhizome de Saposhnikovia divaricata

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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

Saposhnikovia divaricata root and rhizome is the dried root and rhizome of *Saposhnikovia divaricata* (Turcz.) Schischk. It was first recorded in the book *Divine Farmer's Classic of Materia Medica*, a commonly used reference material in various countries. *Saposhnikovia divaricata* root and rhizome has long been used in traditional Chinese medicine for its multiple therapeutic properties (antipyretic, analgesic, sedative, anti-inflammatory, anti-allergic and anti-convulsion). It is a highly regarded traditional Chinese medicine with significant medicinal and economic value around the world. Due to annually increasing domestic and international demand, cultivated herbs have begun to replace wild herbs on the market. The markets of Japan, Korea, Germany, and China, amongst others, have a large circulation of *Saposhnikovia divaricata* root and rhizome.

Saposhnikovia divaricata root and rhizome is mainly distributed in the northeast China (Inner Mongolia, Heilongjiang, Liaoning, Jilin), which are the major production areas of China and account for more than 80 % of national production. It is also planted in Hebei and Shanxi Provinces. The qualities of *Saposhnikovia divaricata* root and rhizome from the producing areas differ in their appearance and chemical contents when comparing cultivated and wild ones. Therefore, the work for identification and quality evaluation of *Saposhnikovia divaricata* root and rhizome is highly important.

Although *Saposhnikovia divaricata* root and rhizome has been recorded in Chinese Pharmacopoeia (2020 edition), Japanese Pharmacopoeia (17th edition), and Korean Pharmacopoeia (10th edition), these standards are not harmonized and can be unsuitable for the purpose of international trade of *Saposhnikovia divaricata* root and rhizome. Therefore, it is of utmost urgency to standardize the quality of *Saposhnikovia divaricata* root and rhizome in the world. This action will benefit consumers (patients), farmers, enterprises and companies related to the planting, management and trade of *Saposhnikovia divaricata* root and rhizome.

As national implementation can 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 D.

Traditional Chinese medicine — *Saposhnikovia divaricata* root and rhizome

1 Scope

This document specifies the quality and safety requirements of *Saposhnikovia divaricata* root and rhizome derived from the plant of *Saposhnikovia divaricata* (Turcz.) Schischk.

It is applicable to *Saposhnikovia divaricata* root and rhizome that is sold as natural medicine 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, *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, *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

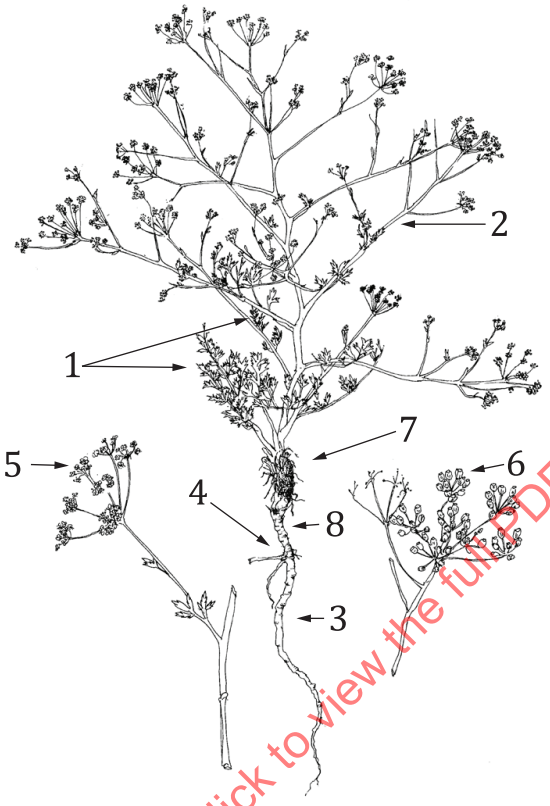
— IEC Electropedia: available at https://www.electropedia.org/

3.1
wild *Saposhnikovia divaricata* root and rhizome
dried root and rhizome of *Saposhnikovia divaricata* (Turcz.) Schischk that grows naturally without cultivation

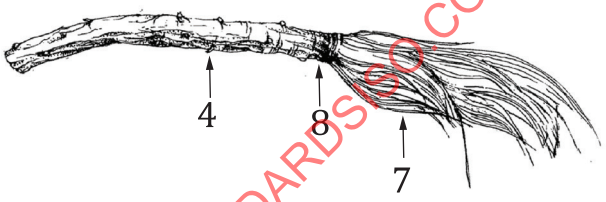
3.2
cultivated *Saposhnikovia divaricata* root and rhizome
dried root and rhizome of *Saposhnikovia divaricata* (Turcz.) Schischk that are artificially cultivated

4 Descriptions

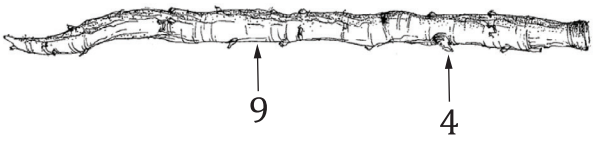
In this document, *Saposhnikovia divaricata* root and rhizome is the dried root and rhizome of *Saposhnikovia divaricata* (Turcz.) Schischk. in the family of *Apiaceae* as shown in Figure 1.



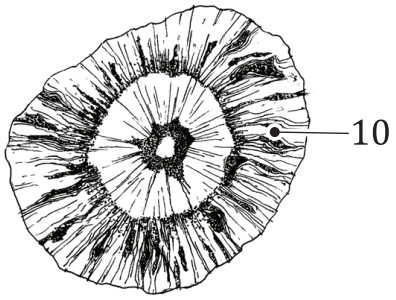
a) plant of *Saposhnikovia divaricata* (Turcz.) Schischk.



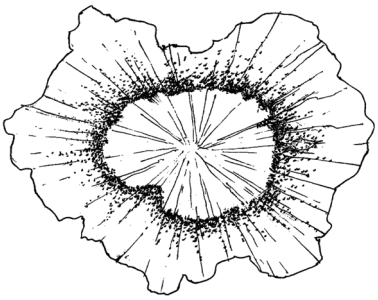
b) wild *Saposhnikovia divaricata* root and rhizome



c) cultivated *Saposhnikovia divaricata* root and rhizome



d) transverse section of wild herb



e) transverse section of cultivated herb

Key

1 leaf
2 stem
3 tap root
4 lateral root
5 inflorescence
6 seed
7 hair-like remains of leaf sheath
8 dense crosswise wrinkles
9 longitudinal wrinkles
10 lacunae

Figure 1 — Structure of *Saposhnikovia divaricata* and *Saposhnikovia divaricata* root and rhizome

5 Requirements

5.1 General characteristics

The following requirements should be met before the sampling.

a) *Saposhnikovia divaricata* root and rhizome should be clean and free from foreign matter.

b) The presence of living insects, mildew and external contaminants which are visible to the naked eye should not be permitted.

5.2 Macroscopic characteristics

5.2.1 Appearance

Long conical or cylindrical rhizome and root, some bend slightly; 15 cm to 30 cm in length, 0,5 cm to 2,0 cm in diameter.

5.2.2 Sensory identification

5.2.2.1 Wild Saposhnikovia divaricata root and rhizome

The surface is pale brown or brown; rhizome reveals dense crosswise wrinkles like ring nodes, and sometimes reveals brown and hair-like remains of leaf sheath; the root reveals many longitudinal

wrinkles and scars of rootlets; in a transverse section, cortex is greyish brown in colour and reveals many lacunae, and xylem is yellow in colour. Odour: slight; taste: slightly sweet.

5.2.2.2 Cultivated *Saposhnikovia divaricata* root and rhizome

The surface is light brown or faint yellow; rhizome's crosswise wrinkles are not distinct. Rhizome sometimes reveals brown and hair-like remains of leaf sheath; the root reveals many longitudinal wrinkles and scars of rootlets; in a transverse section, cortex is pale yellow or brown in colour and reveals few lacunae. Odour: subtle smells; taste: slightly sweet.

5.3 Moisture

The moisture content in percentage mass should not be more than 10,0 %.

5.4 Total ash

The total ash content in percentage mass should not be more than 7,0 %.

5.5 Acid-insoluble ash

The total ash content in percentage mass should not be more than 1,5 %.

5.6 Thin-layer chromatography identification

The identification of *Saposhnikovia divaricata* root and rhizome with thin-layer chromatography (TLC) should present spots or bands obtained from the test and the reference drug solution in the same position with the same colour.

5.7 Dilute ethanol-soluble extractives

The dilute ethanol-soluble extracts content in percentage mass should not be less than 16,0 %.

5.8 Content of marker compound

The contents of marker compounds, such as Prim-*O*-glucosylcimifugin and 5-*O*-methylvisamminoside shall be determined.

5.9 Heavy metals

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

5.10 Pesticide residues

The content of pesticide residues shall be determined.

5.11 Commercial grades

If commercial grades are necessary, see Annex E for additional information.

6 Sampling

Sampling of *Saposhnikovia divaricata* root and rhizome shall be carried out in accordance with ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples not less than 500 g are taken from each batch randomly. These samples are examined by naked-eye observation, smell and taste.

7.2 Determination of moisture content

The testing method specified in ISO 23723 shall apply.

7.3 Determination of total ash

The testing method specified in ISO 23723 shall apply.

7.4 Determination of acid-insoluble ash

The testing method specified in ISO 23723 shall apply.

7.5 Determination of dilute ethanol-soluble extractives content

See Annex A for additional information.

7.6 Thin-layer chromatography (TLC) identification

See Annex B for additional information.

7.7 Determination of maker compound content

See Annex C for additional information.

7.8 Determination of heavy metals content

The testing method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues content

The testing method specified in ISO 22258 shall apply.

8 Test report

For each test sample, the test report should record the sample quantity, sampling date, sale company, as well as producing area and commodity name in detail.

For each test method, the test report shall specify at least the following aspects:

a) all the information necessary for the complete identification of the sample;

b) a reference to this document, including its year of publication (i.e. ISO 23964:2022);

c) the sampling method used;

d) the test method used, with reference to the clause which explains how the results were calculated;

e) the test result(s) obtained;

f) all operating details that are not specified in this document, or regarded as optional, together with details of any deviations or incidents which can have influenced the test result(s);

g) any unusual features (anomalies) observed during the test;

h) the date of the test.

9 Packaging, storage and transportation

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

The storage conditions specified in ISO 22217 shall apply.

Saposhnikovia divaricata root and rhizome should avoid sunlight, rain, moisture and heat during the transportation.

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) the product name and Latin scientific name of the original plant;

b) all quality features indicated in 5.2 to 5.11, determined in accordance with the methods specified in Clause 7;

c) gross weight and net weight of the products;

d) the country and province/state of origin of the product, as well as the name, trademark or logo of the producer and supplier;

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

f) the storage method;

g) items required by regulatory body of the destination country.

Annex A
(informative)

Determination of dilute ethanol-soluble extractives

A.1 Procedure

a) Weigh 250 g of sample to grind and pass it through a 24 mesh or coarse sieve. Weigh approximately 2 g to 4 g (accurate to 0,01 g) of the dried powder into a 250 ml stopper conical flask. Add 50 ml dilute ethanol (50 %) and weigh.

b) Allow the mixture of the powder and dilute ethanol to stand at room temperature for 1 h. Extract with dilute ethanol by refluxing in water-bath (100 °C) for 1 h, cool at room temperature and weigh again.

c) Make up for the lost weight with dilute ethanol and shake well, then filter, evaporate a 25 ml filtrate to dryness, dry at 105 °C for 3 h, and cool in desiccators (silica gel) for 30 mins, then weigh.

A.2 Expression of result

Calculate percentage content of the extract in the sample on the dried basis (%) with Formula (A.1).

$$x = (m_1 - m_0) \times 2 / m_s \times 100 \quad \text{(A.1)}$$

where

x is the dilute ethanol-soluble extractives value (%);

m_1 is the weight of evaporating dish and residue after drying (g);

m_0 is the weight of evaporating dish (g);

m_s is the weight of sample (g).

Annex B
(informative)

Thin layer chromatography (TLC) identification

B.1 Preparation of test solution

Weigh 250 g of *Saposhnikovia divaricata* root and rhizome to grind and pass it through an 80 mesh or finer sieve. Weigh 1 g of the *Saposhnikovia divaricata* root and rhizome samples and add 20 ml of acetone. Ultrasound treat for 20 min, filter, then evaporate to dryness. Then, dissolve the residue with 1 ml of alcohol to create the test solution.

B.2 Preparation of reference solution

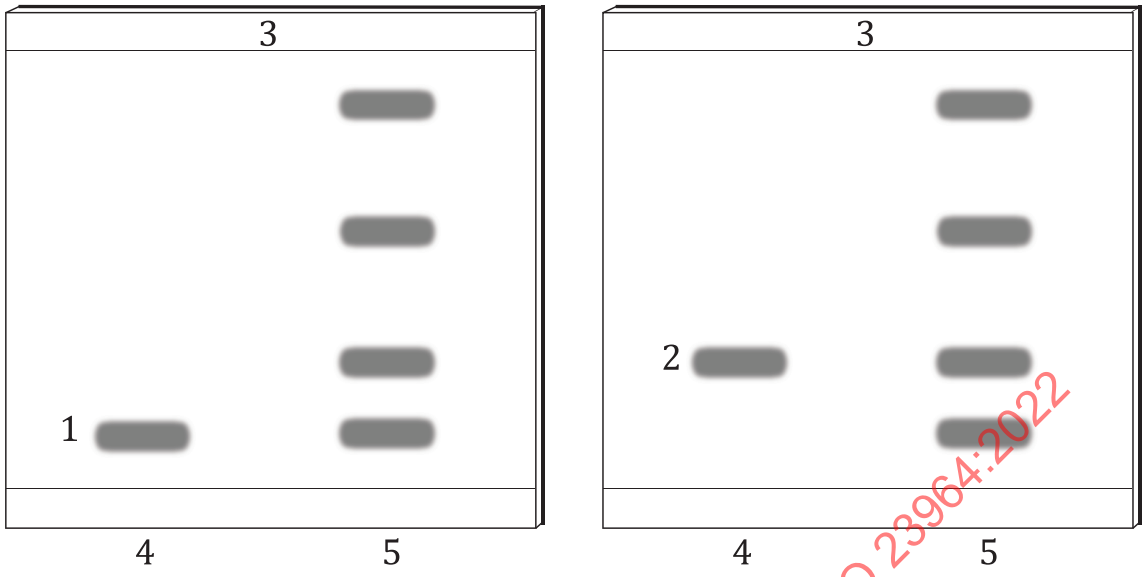
Dissolve Prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside RS in ethyl alcohol separately to produce a solution containing 1 mg per ml as the reference solution.

B.3 Procedure

Carry out the method for thin layer chromatography using silica gel G as the coating substance and a mixture of dichloromethane and methanol (4:1; volume fraction) as the developing solvent system. Separately apply 10,0 µL of the test solution and 10,0 µL of the reference solution to the plate. After developing and removal of the plate, examine under ultraviolet light at 254 nm to obtain the distinct colour of the spots.

B.4 Result

The spots in the chromatograms obtained with the test solution correspond in position and colour to the spots in the chromatograms obtained with the reference solution. Typical reference TLC chromatograms are shown in Figure B.1.



Key

1 5-*O*-methylvisammioside
2 prim-*O*-glucosylcimifugin
3 top of the plate
4 reference solution
5 test solution

Figure B.1 — Schematic diagram of typical TLC chromatogram of *Saposhnikovia divaricata* root and rhizome

Annex C
(informative)

Determination of Prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside content

C.1 Preparation of reference solution

Dissolve a quantity of Prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside RS with methanol in a volumetric flask to prepare two solutions containing 0,06 mg of each per ml as the two reference solutions.

C.2 Preparation of test solution

Weigh 0,25 g of the finely-ground *Saposhnikovia divaricata* root and rhizome and extract with 10 ml of methanol in a conical flask with cover (weigh before placing in water-bath) by refluxing in a water-bath (80 ºC) for 2 h. Cool at room temperature. Weigh the whole weight and make up for the lost weight with methanol. Filter the solution through 0,22 µm millipore filter unit prior to the high performance liquid chromatography (HPLC) analysis.

C.3 Chromatographic system

C.3.1 Column: 4,6 mm × 250 mm; 5 µm (similar to Agilent XDB-C18)

C.3.2 Mobile phase

C.3.2.1 Mobile phase A: acetonitrile

C.3.2.2 Mobile phase B: 0,1 % phosphoric acid in water

C.3.2.3 Isocratic elution: acetonitrile-0,1 % phosphoric acid in water (20:80)

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

C.3.4 Detector: UV 254 nm

C.3.5 Injection size: 10 µl

C.3.6 Column temperature: 30 °C

C.3.7 System suitability

C.3.7.1 Samples: reference solution and test solution

C.3.7.2 Suitability requirements

Resolution: no less than 1,5, test solution.

Tailing factor: no more than 1,5 for prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside peak, reference solution.

Relative standard deviation: no more than 2,0 % for prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside peak, reference solution.

Theoretical plates are above 2 000 calculated with prim-*O*-glucosylcimifugin peak.

C.4 Detection and content calculation

Using the chromatogram of the reference solution and the retention time, identify the retention time of the peak corresponding to prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside in the test solution. Calculate the percentage of prim-*O*-glucosylcimifugin and 5-*O*-methylvisammioside in the portion of *Saposhnikovia divaricata* root and rhizome taken using Formula (C.1):

$$R = (a_U/a_S) \times C_S \times (V/m) \times 10^6 \quad \text{(C.1)}$$

where

R is the result (%);

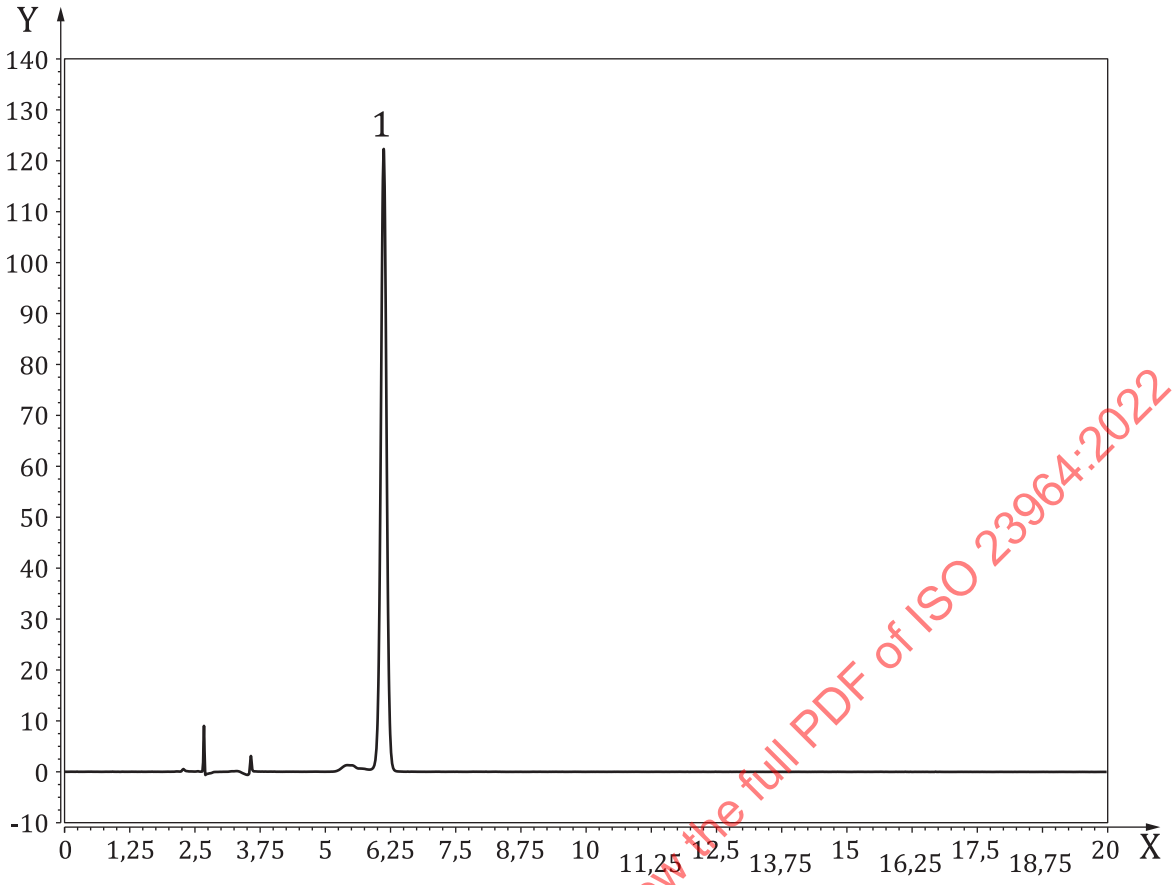
a_U is the peak area of test solution (mAU*min);

a_S is the peak area of prim-*O*-glucosylcimifugin or 5-*O*-methylvisammioside from the reference solution (mAU*min);

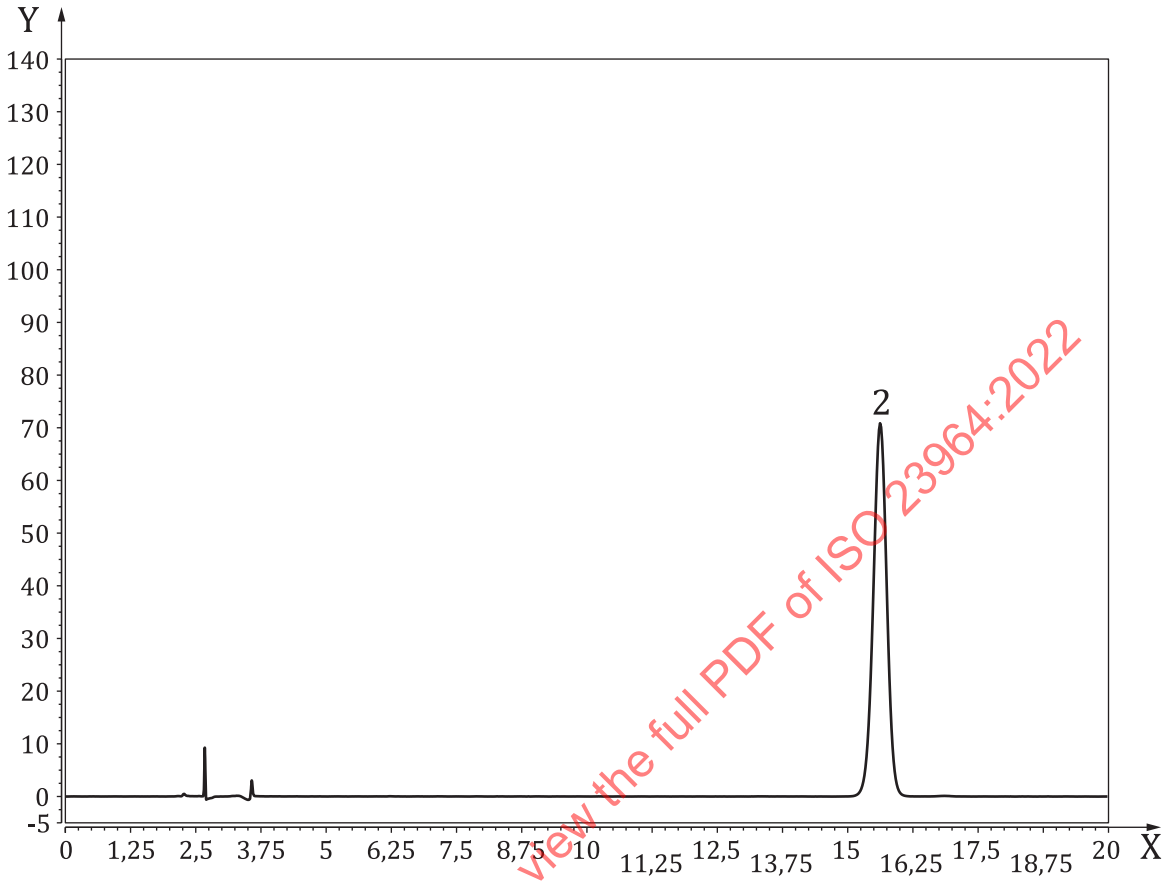
C_S is the concentration of prim-*O*-glucosylcimifugin or 5-*O*-methylvisammioside RS in the reference solution (ml/min);

V is the volume of the test solution (ml);

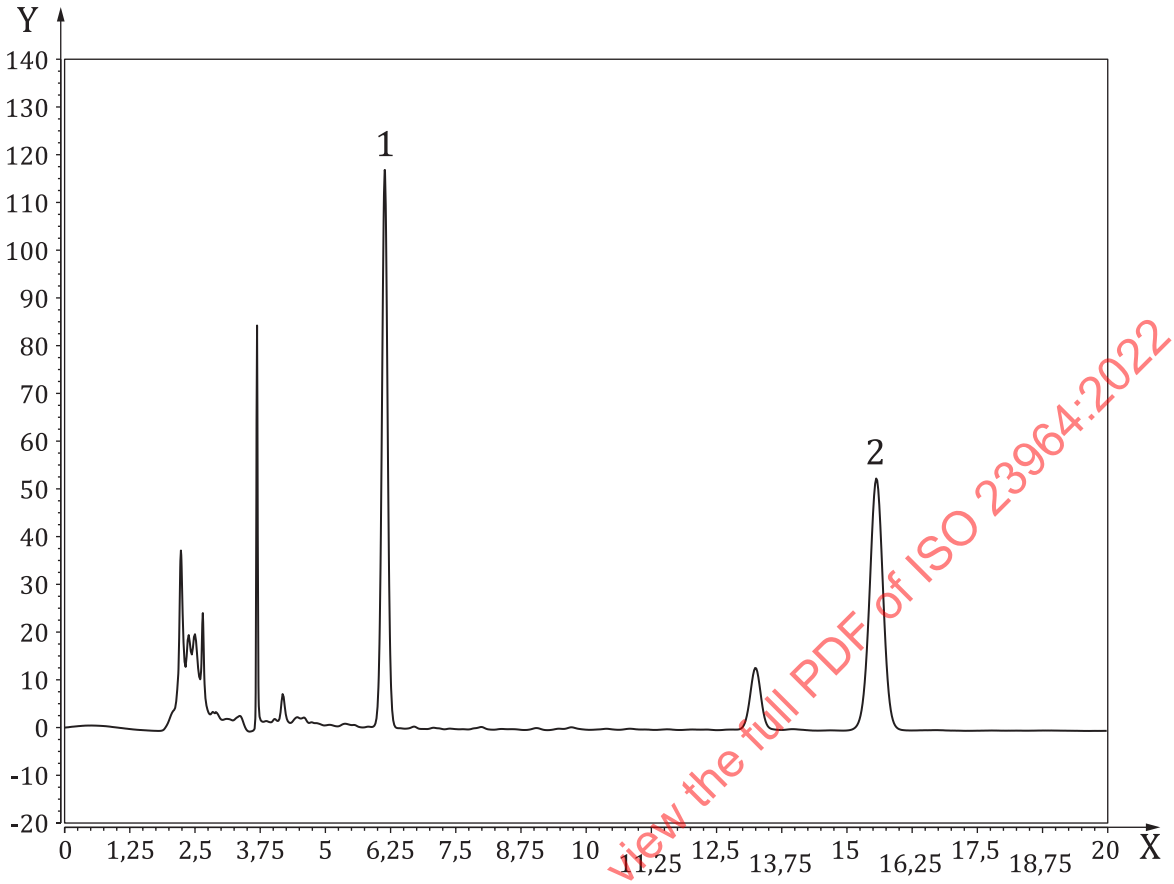
m is the weight of *Saposhnikovia divaricata* root and rhizome taken to prepare the test solution (g).



a) HPLC chromatogram of prim-*O*-glucosylcimifugin reference solution



b) HPLC chromatogram of 5-*O*-methylvisammioside reference solution



c) HPLC chromatogram of *Saposhnikovia divaricata* root and rhizome

Key

X retention time
Y response signal
1 peak of prim-*O*-glucosylcimifugin
2 peak of 5-*O*-methylvisammioside

Figure C.1 — HPLC chromatograms of *Saposhnikovia divaricata* root and rhizome (254 nm)