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Traditional Chinese medicine — *Rehmannia glutinosa* root

Médecine traditionnelle chinoise — Racine de Rehmannia glutinosa

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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

Rehmannia glutinosa root, dried root of *Rehmannia glutinosa* Libosch. (Fam. Scrophulariaceae), is one of the most commonly used herbs in traditional Chinese medicine. It has a long history of use in East and Southeast Asian countries for relieving heat and cooling the blood, and for nourishing yin and body fluid, meaning promoting overall health and body fluid circulation.

Clinically, *Rehmannia glutinosa* root is used for the prevention and treatment of diabetes, inflammation, cancer and other diseases. At present, *Rehmannia glutinosa* root and its processed products occupy a huge share of the international market. The international trading amount of *Rehmannia glutinosa* root ranks in the top ten of Chinese materia medica in countries including China, the Republic of Korea, Japan, Viet Nam and Malaysia. However, many problems can affect the quality of *Rehmannia glutinosa* root, such as different quality requirements among different countries and regions, different preparation methods, and different packaging, transportation and storage conditions. Therefore, the establishment of an International Standard is necessary to meet the quality requirements of *Rehmannia glutinosa* root, supporting its clinical effectiveness and safety.

As national implementation can 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 F.

Traditional Chinese medicine — *Rehmannia glutinosa* root

1 Scope

This document specifies the requirements and test methods for dried *Rehmannia glutinosa* root that is derived from *Rehmannia glutinosa* Libosch.

Fresh *Rehmannia glutinosa* Libosch. and processed *Rehmannia glutinosa* Libosch. are excluded.

This document is applicable to *Rehmannia glutinosa* root that is sold and used as a 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 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23193, *Traditional Chinese medicine — Lycium barbarum and Lycium chinense fruit*

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
main underground part of *Rehmannia glutinos*a Libosch.

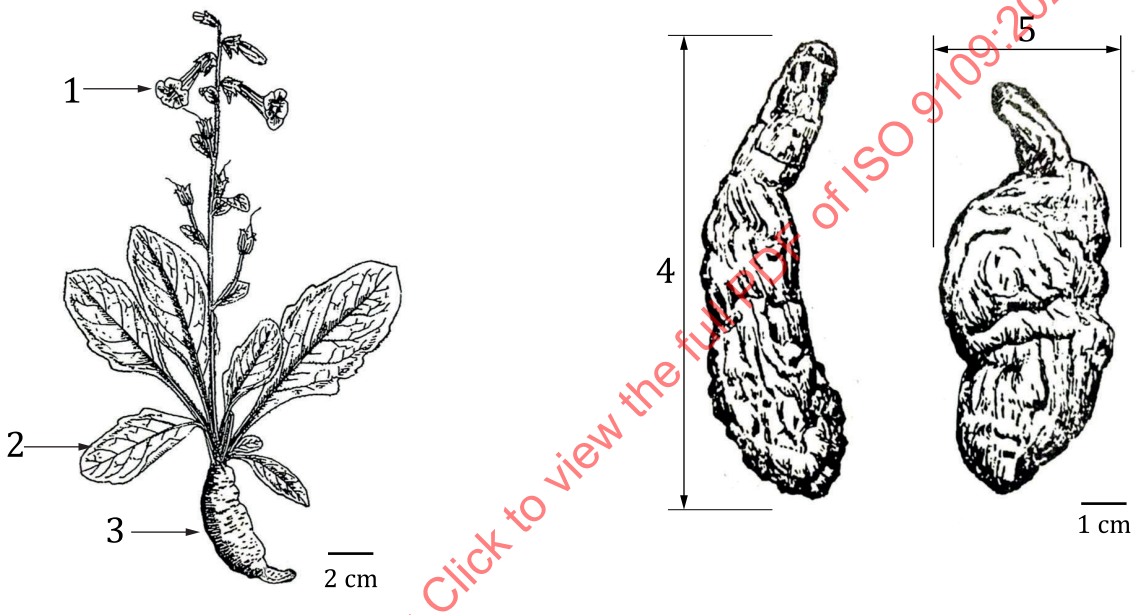
3.2
root weight
average weight of final samples of root, calculated by sampling no less than 1 000 g from each batch randomly, weighing the roots one by one, and then calculating the average weight of the roots

[SOURCE: ISO 20409:2017, 3.2, modified — details on how to calculate root weight have been added.]

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

4 Description

Rehmannia glutinosa root is the dried root of *Rehmannia glutinosa* Libosch. (Fam. Scrophulariaceae), as shown in Figure 1.



a) Plant of *Rehmannia glutinosa* Libosch. **b) Dried root**

Key

1	flower	4	length
2	leaf	5	diameter
3	root		

Figure 1 — Structure of *Rehmannia glutinosa* Libosch.

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Rehmannia glutinosa* root shall be clean and free of foreign matter.

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

5.2 Morphological characteristics

a) The roots are an irregular mass or oblong, swollen in the centre, slightly tapering at both ends; some are small, slat-shaped, slightly compressed or twisted, with an irregular transverse flexure.

b) The external surface is brownish-black or brownish-grey.

c) The root is 6 cm to 12 cm in length and 2 cm to 6 cm in diameter, measured at the base of the root.

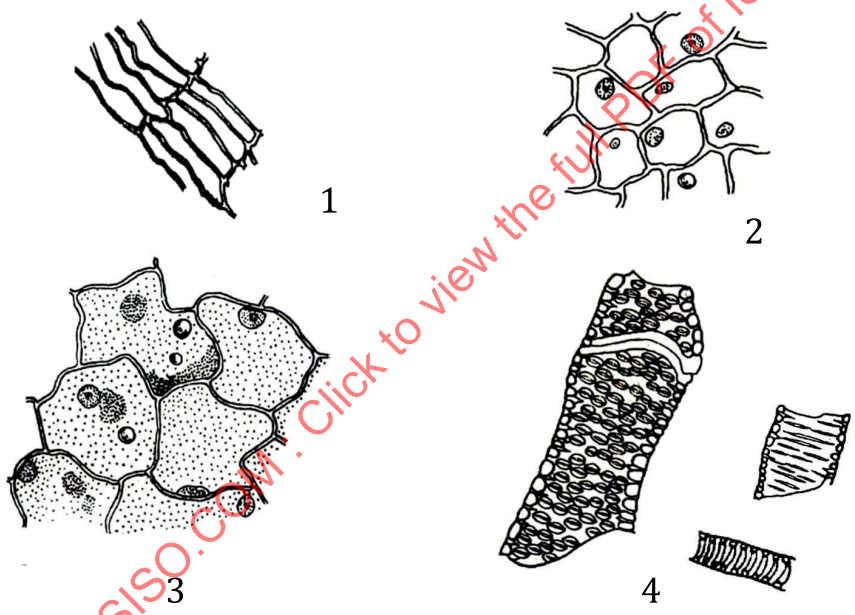
d) The texture is soft and thick, but libriform, not easily broken.

e) The fracture is brownish-yellow to black or jet-black, shiny, with viscosity.

f) The odour is slight and the taste is slightly sweet.

5.3 Microscopic characteristics

The powder is dark brown. Cork cells are brownish. Parenchymatous cells are sub-rounded, containing a sub-rounded nuclei-like substance. Secretory cells are similar to parenchymatous cells in shape, containing orange-yellow or orange-red oil droplets. Bordered pitted and reticulated vessels, shown in Figure 2, are up to about 92 µm in diameter.



Key

1 cork cells
2 parenchymatous cells
3 secretory cells
4 bordered pitted and reticulated vessels

Figure 2 — Structure of powdered *Rehmannia glutinosa* root

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 8,0 %.

5.6 Acid-insoluble ash

The mass fraction of total ash should not be more than 3,0 %.

5.7 Water-soluble extractives

The mass fraction of water-soluble extractives should be determined.

5.8 Thin-layer chromatogram (TLC) identification

The identification of marker compounds such as catalpol and verbascoside with a thin-layer chromatogram (TLC) should present the spots or bands obtained from the test and reference solutions in the same positions with the same colours.

5.9 Content of marker compounds

The content of marker compounds such as catalpol and rehmannioside D should be determined.

5.10 Heavy metals

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

5.11 Pesticide residues

The contents of pesticide residues such as hexachlorocyclohexane, dichlorodiphenyltrichloroethane (DDT) and pentachloronitrobenzene shall be determined.

5.12 Sulfur dioxide residues

The content of sulfur dioxide residues should be determined.

5.13 Grading

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

6 Sampling

Sampling of *Rehmannia glutinosa* root shall be carried out in accordance with the method specified in ISO 23723.

7 Test methods

7.1 Macroscopic identification

Take samples of not less than 1 000 g from each batch randomly. Examine these samples with the naked eye, smell and taste.

7.2 Determination of moisture

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 water-soluble extractives

The testing method specified in ISO 23193 shall apply.

7.6 Thin-layer chromatogram (TLC) identification

See Annex A and Annex B for additional information.

7.7 Determination of marker compounds

See Annex C and Annex D for additional information.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues

The testing method specified in ISO 22258 shall apply.

7.10 Determination of sulfur dioxideresidues

The testing method specified in ISO 22590 shall apply.

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(s) used;

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 could 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 methods specified in ISO 22217 shall apply. Packaging shall not transmit any odour or flavour to the product and shall not contain substances which could damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The *Rehmannia glutinosa* 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 shall apply. 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 or quantity;

d) contact information;

e) name of raw materials;

f) warning statements, if any;

g) expiry date;

h) storage method;

i) batch or lot number;

j) miscellaneous.

Annex A
(informative)

Identification of catalpol

A.1 Preparation of test solution

Weigh 250 g of *Rehmannia glutinosa* root to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 2 g of the powder, add 20 ml of methanol, reflux for 1 h and filter. Concentrate the filtrate to 5 ml as the test solution.

A.2 Preparation of reference solution

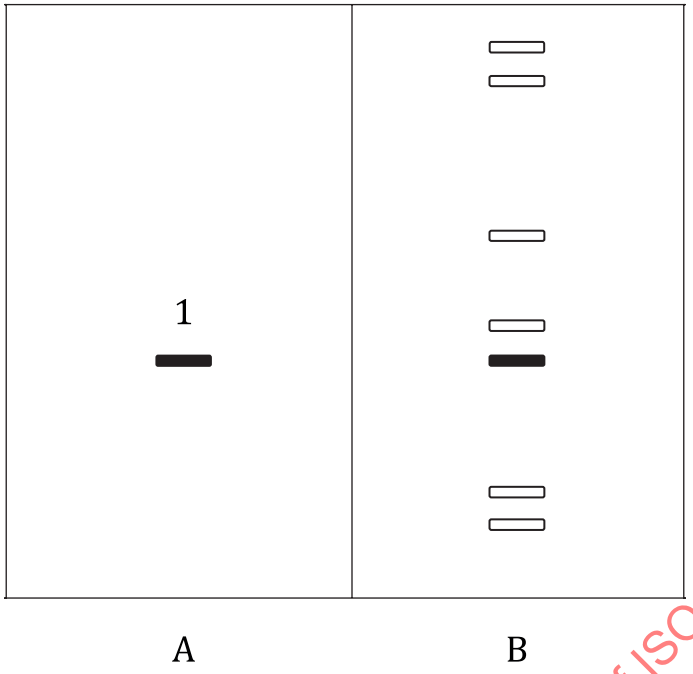
Dissolve reference standards of catalpol in methanol to prepare the reference standard solution of 0,5 mg/ml.

A.3 Developing solvent system

Prepare a mixture of trichloromethane, methanol and water in the volume ratio of 14:6:1 as the mobile phase.

A.4 Procedure

Apply 5 µl each of the reference standard solution and the test solution on the same TLC plate (silica gel G or equivalent plate). Develop the plate in the mobile phase, then take it out and dry in air. Spray p-anisaldehyde solution over the TLC plate and heat at 105 °C until the spots look clear. Identify the spot of catalpol of the test solution by comparing the positions and colours with the reference solution. Typical reference TLC chromatograms are shown in Figure A.1.



Key

1 catalpol
A reference solution
B test solution

Figure A.1 — Schematic diagram of typical TLC chromatogram by catalpol of *Rehmannia glutinosa* root

Annex B
(informative)

Identification of verbascoside

B.1 Preparation of test solution

Weigh 250 g of *Rehmannia glutinosa* 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 and ultrasonicate for 30 min. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of water, then extract four times with 10 ml of water-saturated n-butanol. Combine n-butanol liquids and evaporate the solvent to dryness, then dissolve the residue in 2 ml of methanol as the test solution.

B.2 Preparation of reference solution

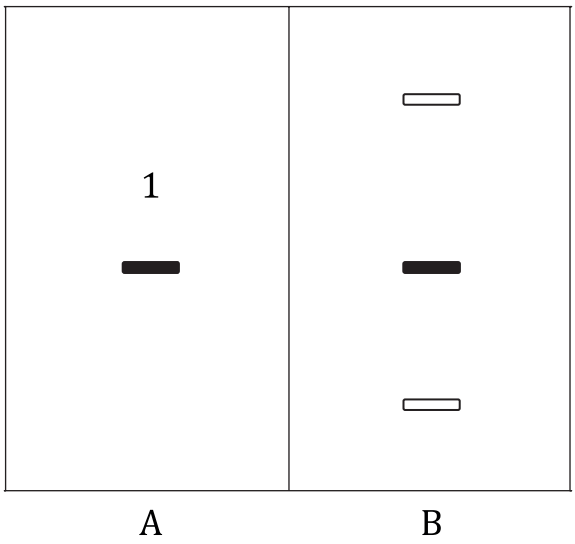
Dissolve reference standard of verbascoside in methanol to prepare the reference standard solution of 1 mg/ml.

B.3 Developing solvent system

Prepare a mixture of ethyl acetate, methanol and formic acid in the volume ratio of 16:0,5:2 as the mobile phase.

B.4 Procedure

Apply 2 µl of the reference standard solution and 5 µl of the test solution on the same TLC plate (silica gel G or equivalent plate). Develop the TLC plate in the mobile phase, then take it out and dry in air. Spray 0,1 % 2,2-diphenyl-1-picrylhydrazyl ethanol absolute solution over the TLC plate and dry in air until the spots look clear. Identify the spot of verbascoside in the test solution by comparing the positions and colours with the reference solution. Typical reference TLC chromatograms are shown in Figure B.1.



Key

1 verbascoside

A reference solution

B test solution

Figure B.1 — Schematic diagram of typical TLC chromatogram by verbascoside of *Rehmannia glutinosa* root

Annex C
(informative)

Determination of catalpol content

C.1 Preparation of reference standard solution

Dissolve a quantity of catalpol with mobile phase to make a solution containing 10 µg per ml as the reference standard solution.

C.2 Preparation of test solution

Take 250 g of *Rehmannia glutinosa* root cut into 5 mm pieces. After 80 °C decompression drying for 24 h, ground into coarse powder and pass through a 24-mesh seive. Take about 0,8 g, weigh precisely, then put into a conical flask with a stopper. Accurately add 50 ml methanol and weigh, then heat reflux for 1,5 h, cool and weigh again. Replenish the loss of solvent with methanol, shake well and filter. Next, take 10 ml of the filtrate and accurately evaporate to dryness. Dissolve the residue in the mobile phase and transfer to a 10 ml volumetric flask. Finally, bring the volume to 10 ml with mobile phase, shake well, filter, and take the filtrate as the test solution.

C.3 Chromatographic system

C.3.1 Column

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

C.3.1.2 Size: I = 250 mm, Ø = 4,6 mm.

C.3.1.3 Theoretical plates: not less than 5 000.

C.3.2 Mobile phase

C.3.2.1 Mobile phase A: methanol.

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

C.3.2.3 Isocratic elution: a mixture of mobile phases A and B (1:99).

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

C.3.4 Detector: 210 nm.

C.3.5 Injection volume: 10 µl.

C.4 Content calculation of catalpol

The content of catalpol, C_{cat} (%), is calculated with Formula (C.1).

$$C_{cat}\,(\%) = \frac{A_1 \times C}{A_2 \times m \times (1 - C_m) \times 200} \times 100\ \% \qquad \text{(C.1)}$$

where

A_1 is the area of the peak due to catalpol ($C_{15}H_{22}O_{10}$) in the chromatogram obtained with the test solution;

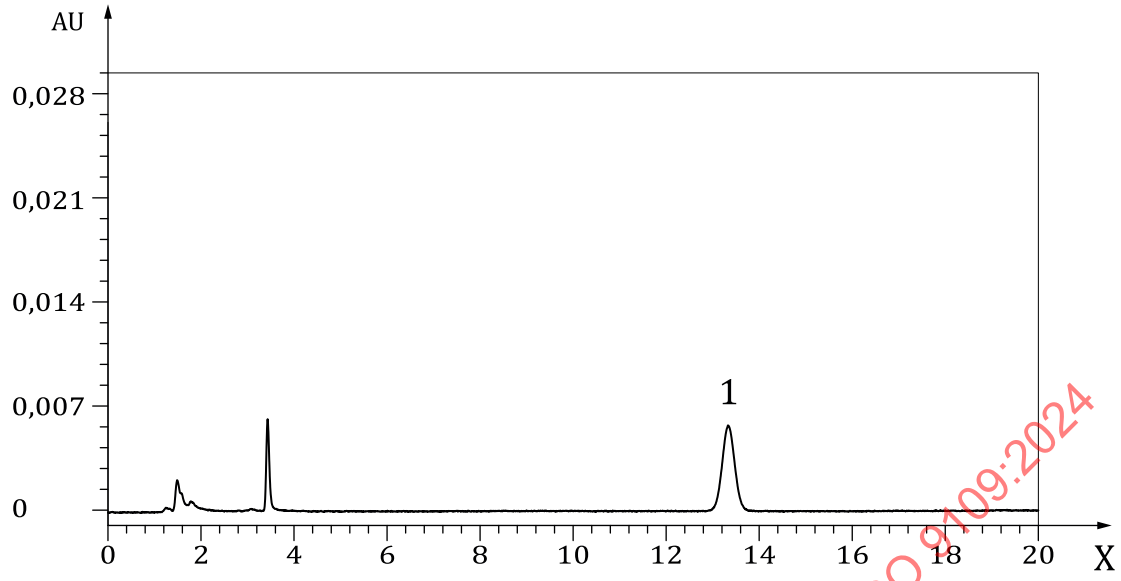
A_2 is the area of the peak due to catalpol ($C_{15}H_{22}O_{10}$) in the chromatogram obtained with the reference solution;

C is the density of catalpol in the reference solution (µg/ml);

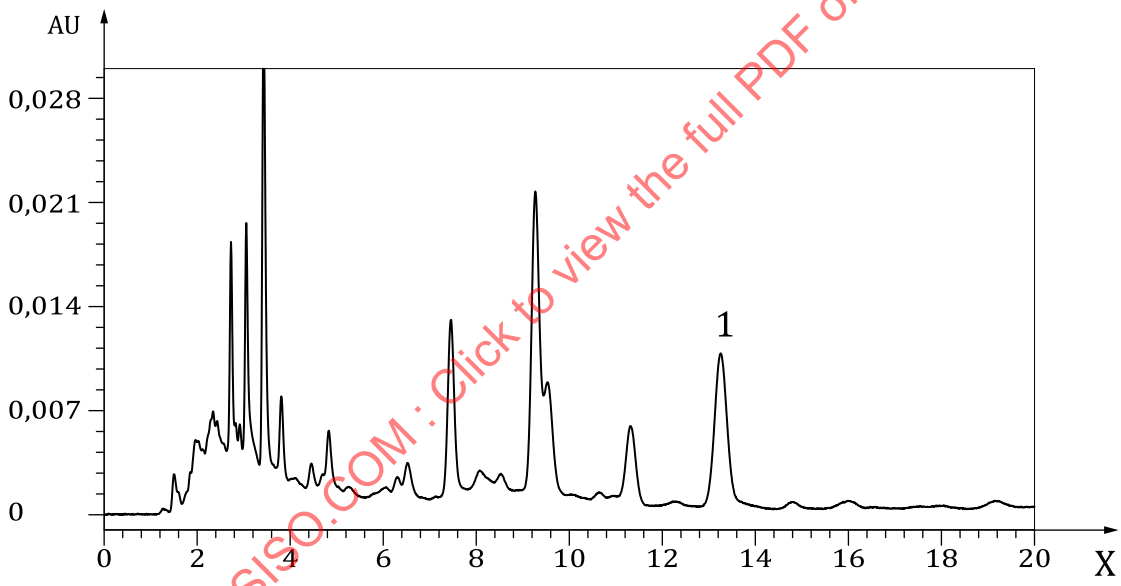
m is the mass of the sample to be examined used to prepare the test solution (g);

C_m is the percentage moisture content of the sample.

A typical reference HPLC chromatogram is shown in Figure C.1.



a) Reference standard solution



b) Sample solution

Key

X min

1 catalpol

Figure C.1 — Typical reference HPLC chromatogram by catalpol of *Rehmannia glutinosa* root

Annex D
(informative)

Determination of rehmannioside D content

D.1 Preparation of reference standard solution

Dissolve a quantity of rehmannioside D with 25 % methanol to make a solution containing 70 µg per ml as the reference standard solution.

D.2 Preparation of test solution

Take 250 g of *Rehmannia glutinosa* root cut into 5 mm pieces. After 80 °C decompression drying for 24 h, ground into meal and pass it through a 24-mesh sieve. Take about 1 g, weigh precisely, then put into a conical flask with a stopper. Accurately add 25 ml 25 % methanol, weigh, ultrasonicate for 1 h, cool and weigh again. Replenish the loss of solvent with 25 % methanol, shake well, centrifuge at high speed for 10 min, take the supernatant and filter. Take the filtrate as the test solution.

D.3 Chromatographic system

D.3.1 Column

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

D.3.1.2 Size: I = 250 mm, Ø = 4,6 mm.

D.3.1.3 Theoretical plates: not less than 5 000.

D.3.2 Mobile phase

D.3.2.1 Mobile phase A: methanol.

D.3.2.2 Mobile phase B: 0,1 % volume fraction of phosphoric acid in water.

D.3.2.3 Isocratic elution: a mixture of mobile phases A and B (5:95).

D.3.3 Flow rate: 1,2 ml/min.

D.3.4 Detector: 203 nm.

D.3.5 Injection volume: 20 µl.

D.4 Content calculation of rehmannioside D

The content of rehmannioside D, C_{rehD} (%), is calculated with Formula (D.1).

$$C_{\mathrm{rehD}}\,(\%) = \frac{A_1 \times C}{A_2 \times m \times (1 - C_{\mathrm{m}}) \times 400} \times 100\ \% \qquad \text{(D.1)}$$

where

A_1 is the area of the peak due to rehmannioside D ($C_{27}H_{42}O_{20}$) in the chromatogram obtained with the test solution;

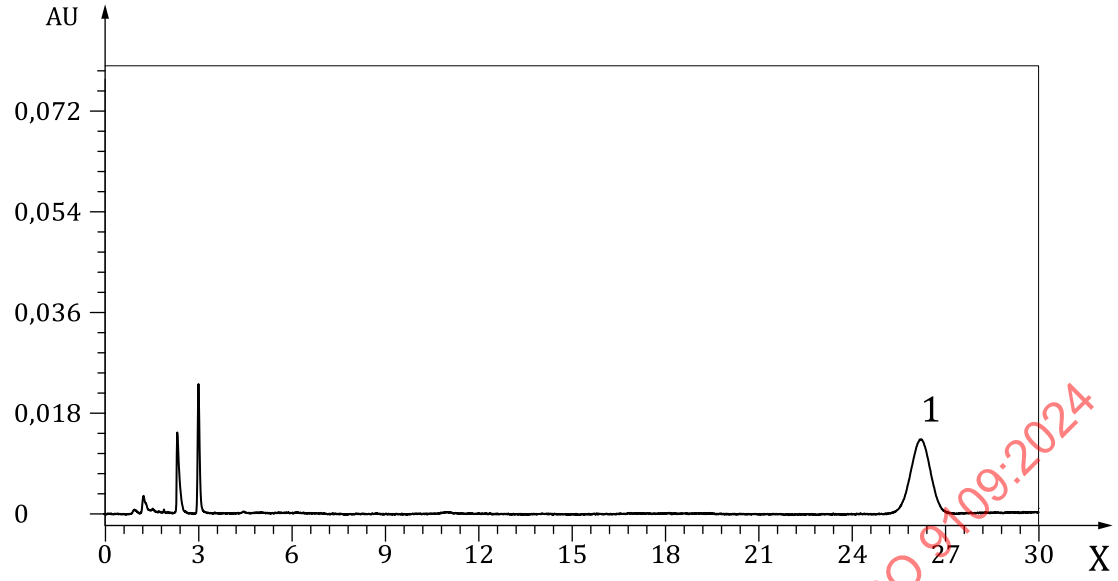
A_2 is the area of the peak due to rehmannioside D($C_{27}H_{42}O_{20}$) in the chromatogram obtained with the reference solution;

C is the density of rehmannioside D in the reference solution (µg/ml);

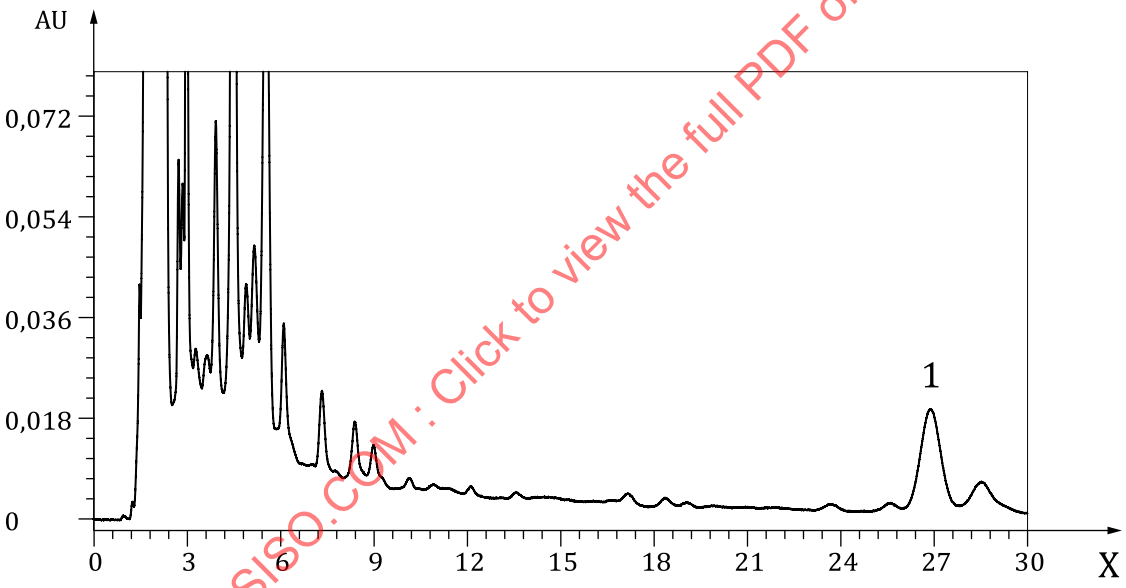
m is the mass of the sample to be examined used to prepare the test solution (g);

C_m is the percentage moisture content of the sample.

A typical reference HPLC chromatogram is shown in Figure D.1.



a) Reference standard solution



b) Sample solution

Key

X min

1 rehmannioside D

Figure D.1 — Typical reference HPLC chromatogram by rehmannioside D of *Rehmannia glutinosa* root