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Traditional Chinese medicine — *Rheum palmatum*, *Rheum tanguticum* and *Rheum officinale* root and rhizome

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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 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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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

Rheum root and rhizome is the dry root and rhizome of *Rheum palmatum* Linne., *Rheum tanguticum* Maxim. ex Balf. and *Rheum officinale* Baill. As one of the four traditional Chinese medicines in China, *Rheum* root and rhizome is documented in *Shennong materia medica*. *Rheum* root and rhizome has a wide range of clinical applications, high frequency of use and a long history of medicinal use. It is recorded in traditional Chinese medicine books such as *Yao pin hua yi*, *Yi xue zhong zhong can xi lu* and *Su Wen*.

Rheum root and rhizome has a complex chemical composition, including various compounds such as anthraquinone, anthrone, tannin and polysaccharide. Modern pharmacological studies have shown that sennoside and anthraquinone glucosides are the main components of *Rheum* root and rhizome that can induce diarrhoea; free anthraquinones are antibacterial and antitumour active ingredients of *Rheum* root and rhizome; n-butyrophenones have good anti-inflammatory and analgesic effects; gallic acid glucosides and galloyl proanthocyanidins in the tannins have hypolipidemic effects; and d-catechin and gallic acid have haemostatic effects. Moreover, *Rheum* root and rhizome also has the functions of relieving phlegm, protecting the liver and gallbladder, and protecting against cardiovascular and cerebrovascular diseases.

In global trade, taking Chinese customs data as an example, *Rheum* root and rhizome in China is mainly exported to Japan, the Republic of Korea, the United States, Indonesia, Germany, Italy, Singapore, France, Thailand, Vietnam and Malaysia. From 2012 to 2016, the average annual export trade volume of *Rheum* root and rhizome in China was 7 128 400 US dollars, making it one of the main Chinese herbal medicines exported by China.

There are 152 Chinese patent medicines containing *Rheum* root and rhizome in the Chinese Pharmacopoeia (2015 Edition) and two preparations in Japanese Pharmacopoeia (17th Edition). *Rheum* root and rhizome and its products have applications in a variety of medical fields, also involving detoxification and beauty, lipid-lowering, weight-loss health products and food additives. Many users believe that Chinese medicine is non-toxic and fail to take it strictly according to their doctor's instructions, which can lead to excessive and chronic irregular use of *Rheum* root and rhizome and liver and kidney damage.

As a globally used drug, *Rheum* root and rhizome is included in the pharmacopoeia of many countries and regions, such as China, Japan, the Republic of Korea and Europe. At present, the quality control of *Rheum* root and rhizome and its preparations is mostly based on the content of anthraquinones. However, the medicinal ingredients of *Rheum* root and rhizome are not just anthraquinones. The diversity of ingredients in traditional Chinese medicine determines that quality control should adopt a multi-index quality evaluation model.

Furthermore, *Rheum* root and rhizome is ranked tenth in the priority list of single herbal medicines for developing standards in ISO/TR 23975, which indicates its high priority. Therefore, it is necessary to establish an International Standard of *Rheum* root and rhizome which unifies the quality and safety of *Rheum* root and rhizome, ensures the safety and effectiveness of the medication and regulates trade in the international market. The establishment of an International Standard for *Rheum* root and rhizome is necessary to guarantee the clinical effectiveness, safety and controllability of this valuable medicine in global commerce and trade.

As national implementation can differ, national standards bodies are invited to modify the values given in 5.5, 5.6, 5.7 and 5.8 in their national standards. Examples of national and regional values are given in Annex C.

Traditional Chinese medicine — *Rheum palmatum*, *Rheum tanguticum* and *Rheum officinale* root and rhizome

1 Scope

This document specifies the quality and safety requirements of *Rheum* root and rhizome (the dried root and rhizome of *Rheum palmatum* Linne., *Rheum tanguticum* Maxim. ex Balf. and *Rheum officinale* Baill.).

This document applies to *Rheum* 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 these plants.

This document does not apply to the processing methods and processed products of *Rheum* root and rhizome.

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/

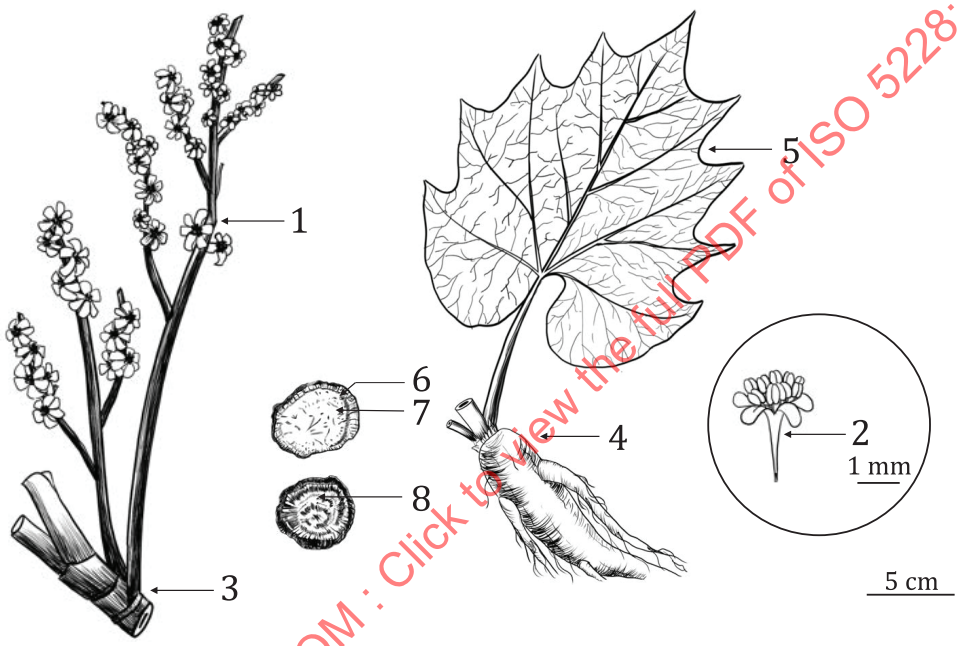
3.1
***Rheum* root and rhizome**
dried root and rhizome of *Rheum palmatum* Linne., *Rheum tanguticum* Maxim. ex Balf. and *Rheum officinale* Baill

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

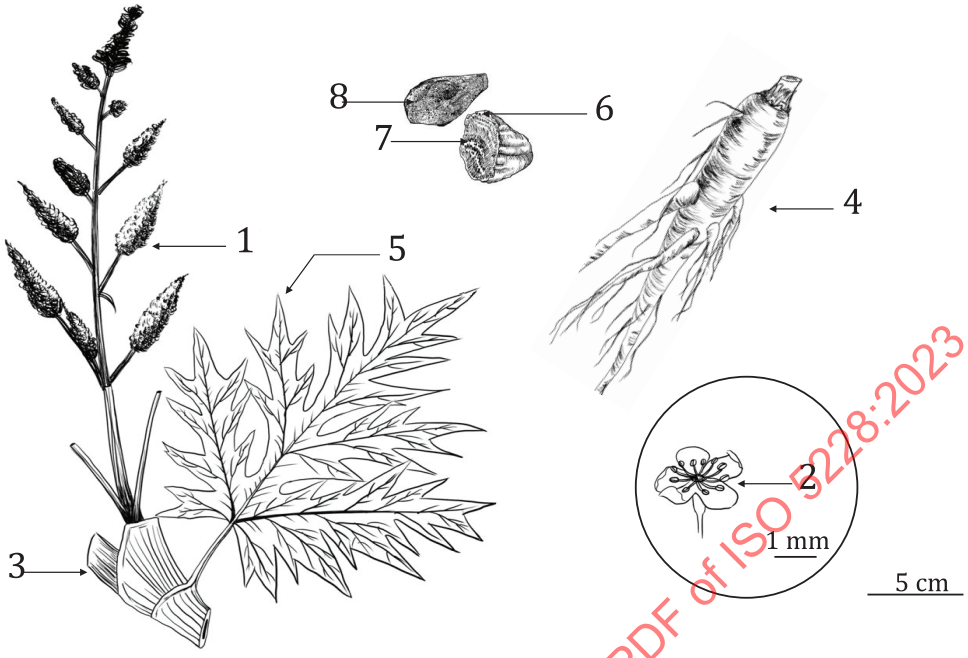
[SOURCE: ISO 22988:2020, 3.8]

4 Description

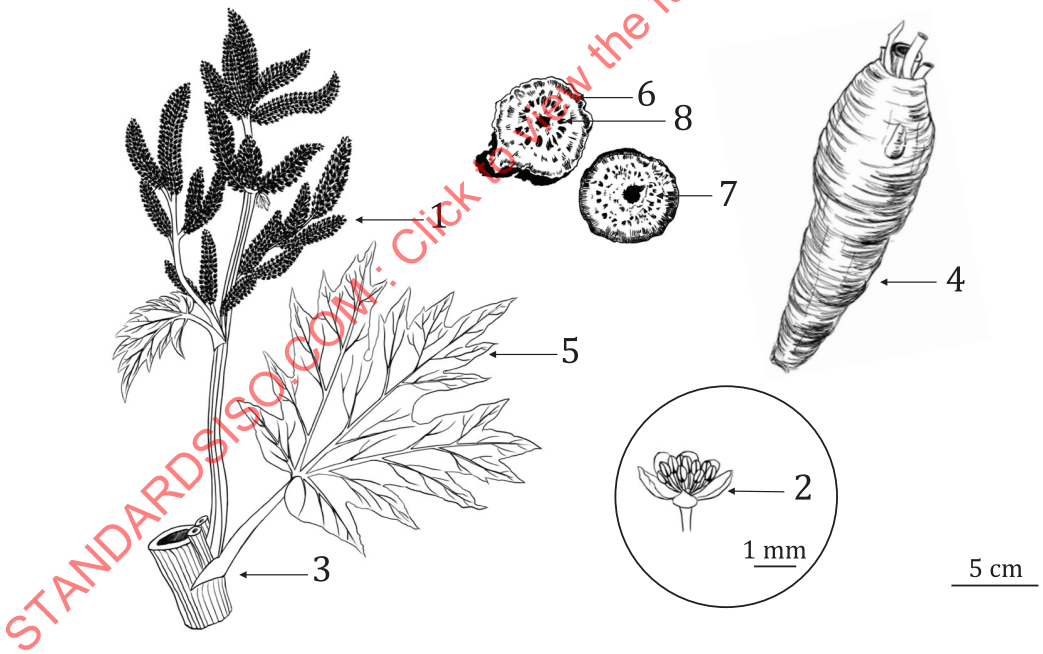
Rheum root and rhizome is the dried root and rhizome of *Rheum palmatum* Linne. (Figure 1 a), *Rheum tanguticum* Maxim. ex Balf. (Figure 1 b) and *Rheum officinale* Baill. (Figure 1 c). The crude drug is collected in late autumn when stem and leaves have withered or in spring just before budding. The crude drug is removed from rootlet and the outer bark, cut into segments or sections and either hung in line for drying or dried directly.



a) *Rheum palmatum* Linne.



b) *Rheum tanguticum* Maxim. ex Balf.



c) *Rheum officinale* Baill.

Key

1	inflorescence	5	leaf
2	flower	6	xylem, cambium and phloem
3	stem	7	pith
4	root and rhizome	8	abnormal vascular bundles

Figure 1 — Structure of *Rheum* root and rhizome

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Rheum* root and rhizome shall be clean and free from leaf and foreign matter.

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

5.2 Morphological features

The medicinal materials of *rheum* root and rhizome are subcylindrical, conical, ovoid or irregular pieces, 3 cm to 17 cm long and 3 cm to 10 cm in diameter. Externally, they are yellowish-brown to reddish-brown when peeled, sometimes with whitish reticulations and visible scattered star spots (abnormal vascular bundles), occasionally with brownish-black patches of cork, mainly with a hole through which the string is passed and coarse wrinkles. The texture of the medicinal parts is firm, sometimes loose and soft in the centre, the facture pale reddish-brown or yellowish-brown and granular. The pith of the rhizome is broad, with star spots arranged in a ring or irregularly scattered. The wood of the root is well developed, lined radially, the cambium ring distinct and without star spots. The odour is delicately aromatic, the taste bitter and slightly astringent. It is sticky and gritty to chew.

NOTE The hole and the string are for hanging *Rheum* root and rhizome when dry.

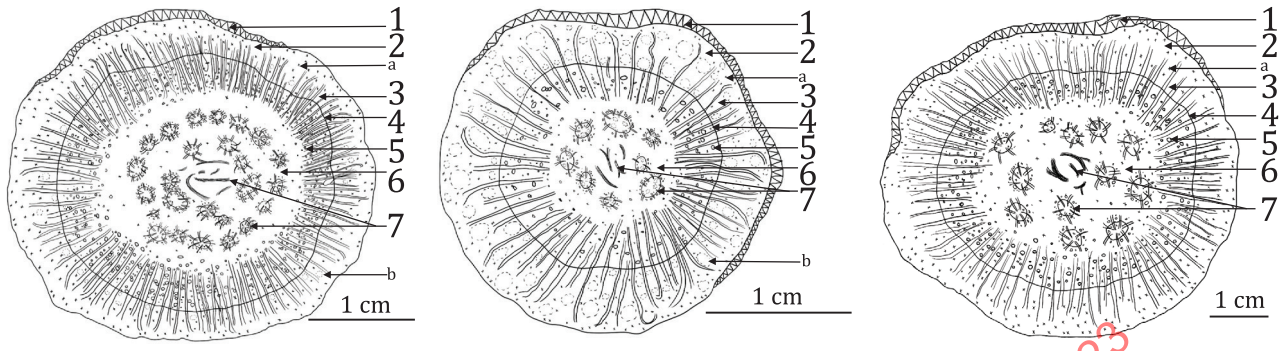
5.3 Identification

5.3.1 Microscopic identification

Transverse section of *Rheum palmatum* Linne. (Figure 2 a), *Rheum tanguticum* Maxim. ex Balf. (Figure 2 b) and *Rheum officinale* Baill. (Figure 2 c): the cork and phelloderm of root are mostly removed. In phloem, the sieve tube groups are distinct and the parenchyma is well developed. The cambium forms a ring. Xylem rays are relatively dense, two to four rows of cells wide, containing brown masses. The vessels are non-lignified, usually single or several grouped together and sparsely arranged. Parenchymatous cells contain clusters of calcium oxalate and abundant starch granules.

The rhizome pith is broad, usually showing mucilage cavities and containing reddish-brown masses, scattered with abnormal vascular bundles. The cambium forms a ring. Xylem is on the inside of the cambium and phloem on the outside. Rays are stellate.

The powder is yellowish-brown. Clusters of calcium oxalate are 20 µm to 160 µm in diameter, sometimes up to 190 µm in diameter. Bordered pitted vessels, reticulated vessels, spiral vessels and annular vessels are non-lignified. Starch granules are abundant, single granules are spheroid or polygonal, 3 µm to 45 µm in diameter, and hilum is stellate. The compound granules consist of two to eight components.



a) *Rheum palmatum* Linne. **b) *Rheum tanguticum* Maxim. ex Balf.** **c) *Rheum officinale* Baill.**

Key

1 cork
2 cortex
3 phloem
4 cambium
5 xylem
6 pith
7 abnormal vascular bundles
[a] Clusters of calcium oxalate.
[b] Mucilage cavities.

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

5.3.2 Thin-layer chromatography (TLC) identification

Spots in the chromatogram obtained with the test solution should correspond in position and colour to the spots in the chromatogram obtained from the reference drug solution or reference solution.

5.4 Rhaponticin

a) The bright-blue fluorescence spot in the chromatogram obtained with the test solution shall not correspond in position and colour to the spot in the chromatogram obtained with the reference solution TLC chromatogram.

b) When high-performance liquid chromatography (HPLC) is performed, the chromatographic peak of the test solution shall not correspond at the retention time to the rhaponticin ($C_{21}H_{24}O_9$) chemical reference standard (CRS) of the HPLC chromatogram.

5.5 Moisture

The content of water should be a mass fraction of 15,0 %.

5.6 Total ash

The content of total ash should be a mass fraction of ≤ 13,0 %.

5.7 Acid-insoluble ash

The content of acid-insoluble ash should be a mass fraction of ≤ 2,0 %.

5.8 Extractives

The content of water-soluble extractives should be a mass fraction of ≥ 25,0 %.

5.9 Heavy metals

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

5.10 Pesticide residues

The contents of pesticide residues shall be determined.

5.11 Marker compounds

The contents of marker compounds, such as total anthraquinone, free anthraquinone and sennoside A, 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

Samples of not less than 500 g are taken from each batch randomly. These samples are examined by the naked eye in sunlight and for smell. The ultra-performance liquid chromatography (UPLC) method specified in Annex D is used to distinguish the three species of *Rheum* root and rhizome.

7.2 Thin-layer chromatogram (TLC) identification

See Clause A.1 for additional information.

7.3 Rhaponticin identification

See Clause A.2 and Clause B.4 for additional information. If the results of the two methods are inconsistent, TLC shall prevail.

7.4 Determination of moisture

The testing method specified in ISO 23723: 2021, 7.2.1 applies.

7.5 Determination of total ash

The testing method specified in ISO 23723: 2021, 7.2.3 applies.

7.6 Determination of acid-insoluble ash

The testing method specified in ISO 23723: 2021, 7.2.3 applies.

7.7 Determination of extractives

The testing method specified in ISO 23723:2021, 7.2.5 applies.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 applies.

7.9 Determination of pesticide residues

The testing method specified in ISO 22258 applies.

7.10 Determination of marker compounds

See Clauses B.1, B.2 and B.3 for additional information.

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, i.e. ISO 5228:2023;

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 packaging and transportation shall not transmit any odour or flavour to the product and shall not contain substances that could damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The storage condition specified in ISO 22217:2020, 5.2.1 shall apply.

The products shall be protected from light, moisture, pollution and foreign substances during long-distance delivery. Carriers should be well ventilated so they remain dry and moisture-proof.

10 Marking and labelling

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

a) all quality features indicated in Clause 5, determined in accordance with methods specified in Clause 7;

b) gross weight and net weight of the package;

c) country, province or state of origin of the products;

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

e) storage method;

f) any items required by the destination.

Annex A
(informative)

Thin-layer chromatography (TLC) identification

A.1 TLC identification of *Rheum* root and rhizome

a) Macerate 0,1 g of the powder in 20 ml of methanol for 1 h and gravity filter. Evaporate 5 ml of the filtrate to dryness, dissolve the residue in 10 ml of water, add 1 ml of hydrochloric acid, heat under reflux on a water bath for 30 min and cool immediately. Extract by shaking with 20 ml of ether twice, combine the ether extracts, evaporate to dryness and dissolve the residue in 1 ml of chloroform as the test solution.

b) Prepare a solution of *Rheum* root and rhizome reference drug in the same manner as the reference drug solution.

c) Dissolve rhein CRS in methanol to produce a solution containing 1 mg/ml as the reference solution.

d) Use the upper layer of a mixture of petroleum ether (30 °C to 60 °C), ethyl formate and formic acid (15:5:1) as the mobile phase.

e) Use silica gel H mixed with sodium carboxymethylcellulose as the coating substance and apply 4 µl each of the test solution, the reference drug solution and the reference solution to the plate. After developing and removing the plate, dry in air and examine under ultraviolet light at 365 nm.

f) The five orange fluorescent spots in the chromatogram obtained with the test solution correspond in position and colour to the spots in the chromatogram obtained with the reference drug solution. The spot becomes red on exposure to ammonia vapour.

A.2 TLC identification of rhaponticin

a) Take 0,1 g of the powder, add 10 ml of methanol, ultrasonicate for 20 minutes and filter. Take 1 ml filtrate and add methanol to 10 ml as the test solution.

b) Dissolve rhaponticin CRS in methanol to produce a solution containing 10 µg/ml as the reference solution (freshly prepared).

c) Use a mixture of toluene, ethyl formate, acetone, methanol and formic acid (30:5:5:20:0,1) as the mobile phase.

d) Use polyamide as the coating substance and apply separately to the film 5 µl each of the test solution and the reference solution. After developing and removing the film, dry in air and examine under ultraviolet light at 365 nm.

e) The bright-blue fluorescence spot in the chromatogram obtained with the test solution shall not correspond in position and colour to the spot in the chromatogram obtained with the reference solution.

Annex B
(informative)

Determination of marker compounds by HPLC-UV

B.1 Determination of total anthraquinone

B.1.1 Preparation of test solution

Weigh accurately 0,15 g of the powder (through no. 4 sieve) to a stoppered conical flask, accurately add 25 ml of methanol and weigh. Heat under reflux on a water bath for 1 h, cool, weigh again, replenish the loss of solvent with methanol, mix well and filter. Measure accurately 5 ml of successive filtrate in a flask, evaporate the solvent, add 10 ml 8 % solution of hydrochloric acid, ultrasonicate for 2 min and add 10 ml of chloroform. Heat under reflux for 1 h, cool, transfer to a separating funnel, wash the flask with a small quantity of chloroform and combine the washings to the separating funnel. Separate the chloroform layer, extract the acid solution again with 10 ml of chloroform three times, combine the chloroform extracts and recover the chloroform in a vacuum to dryness. Dissolve the residue in methanol and transfer to a 10 ml volumetric flask, dilute with methanol to volume and mix well as the test solution.

B.1.2 Preparation of reference solution

Separately weigh quantities of aloe-emodin CRS, rhein CRS, emodin CRS, chrysophanol CRS and physcion CRS accurately, then dissolve each in methanol to produce five solutions containing 80 µg/ml of aloe-emodin, 80 µg/ml of rhein, emodin, 80 µg/ml of chrysophanol and 40 µg/ml of physcion. Measure 2 ml of each solution accurately to produce a 10 ml mixture containing 16 µg/ml of aloe-emodin, rhein, emodin and chrysophanol and 8 µg/ml physcion as the reference solution.

B.1.3 Chromatographic conditions

a) Stationary phase: octadecylsilane bonded silica gel.

b) Column size: 4,6 µm, 5 mm × 150 mm.

c) Mobile phase A: a mixture of methanol and 0,1 % phosphoric acid (85:15).

d) Flow rate: 1 ml/min.

e) Injection volume: 10 µl.

f) Temperature of column oven: 30 °C.

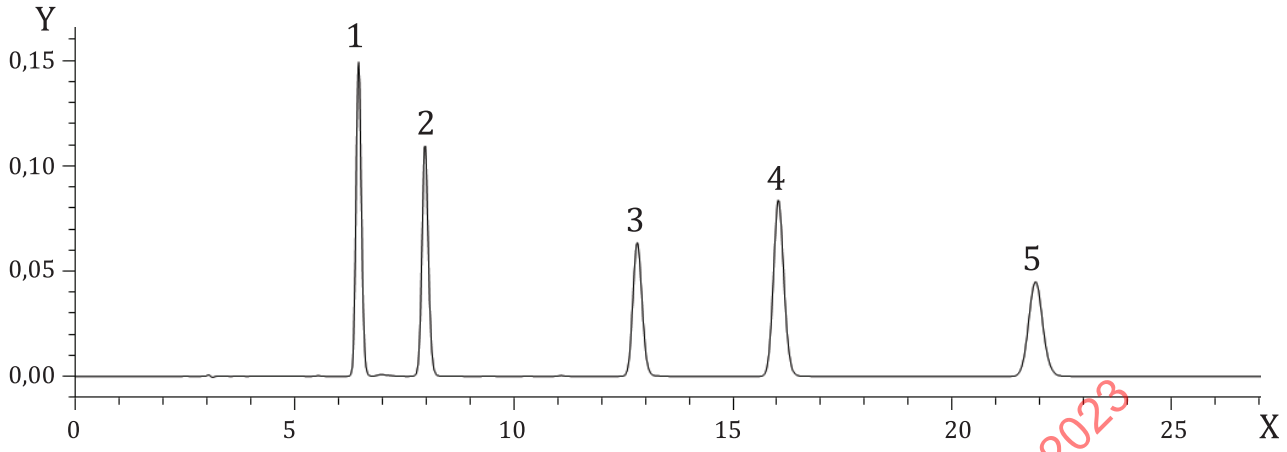
g) Detector: a spectrophotometer set at 254 nm.

B.1.4 Determination

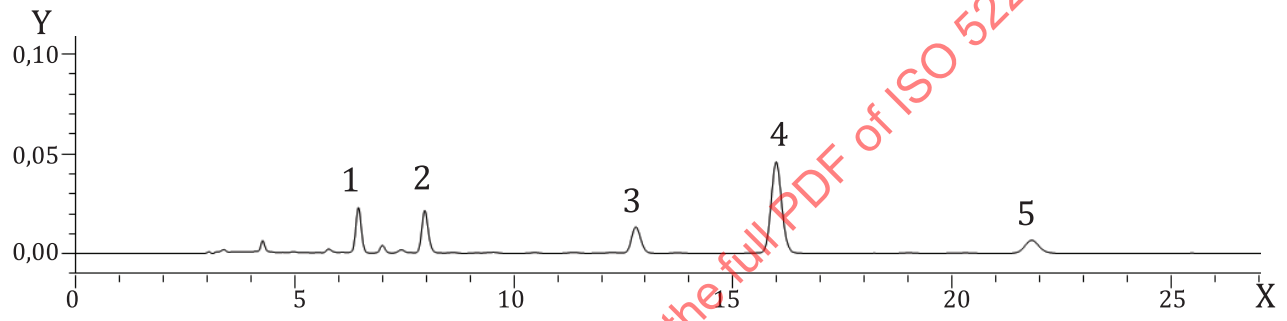
Accurately inject 10 µl each of the reference solution and the test solution into the column and calculate the content. HPLC chromatograms of the reference solution, free anthraquinone of *rheum* root and rhizome sample and total anthraquinone of *rheum* root and rhizome sample are shown in Figure B.1.

The contents of the total anthraquinone amount of aloe-emodin ($C_{15}H_{10}O_5$), rhein ($C_{15}H_8O_6$), emodin ($C_{15}H_{10}O_5$), chrysophanol ($C_{15}H_{10}O_4$) and physcion ($C_{16}H_{12}O_5$) are calculated with reference to the dried drug.

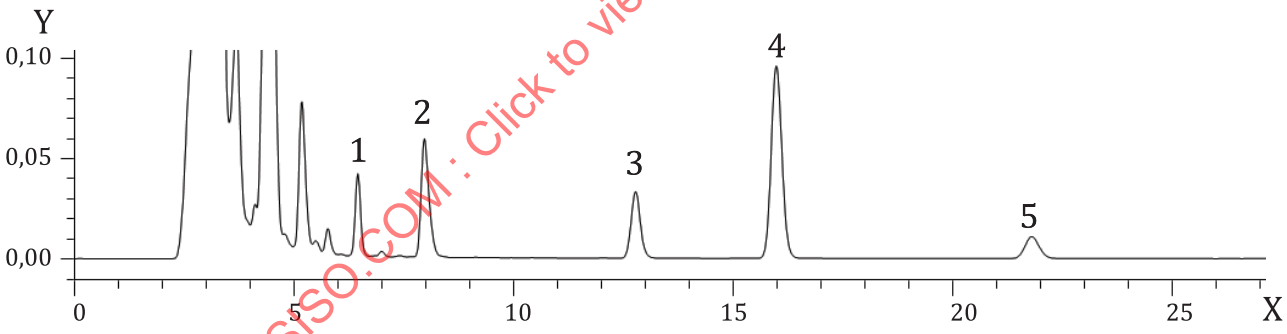
Validate the methodology for accuracy, precision and repeatability, if necessary.



a) Reference solution



b) Free anthraquinone of *rheum* root and rhizome sample



c) Total anthraquinone of *rheum* root and rhizome sample

Key

X mAU
Y min
1 aloe-emodin
2 rhein
3 emodin
4 chrysophanol
5 physcion

Figure B.1 — HPLC chromatograms of *Rheum* root and rhizome

B.2 Determination of free anthraquinone

B.2.1 Preparation of test solution

Weigh accurately 0,5 g of the powder (through no. 4 sieve) to a stoppered conical flask, accurately add 25 ml of methanol and weigh. Heat under reflux on a water bath for 1 h, cool, weigh again, replenish the loss of solvent with methanol, mix well and filter. Use the successive filtrate as the test solution.

B.2.2 Preparation of reference solution

See B.1.2.

B.2.3 Chromatographic conditions

See B.1.3.

B.2.4 Determination

Accurately inject 10 µl each of the reference solution and the test solution into the column and calculate the content.

The content of the free anthraquinone amount of aloe-emodin ($C_{15}H_{10}O_5$), rhein ($C_{15}H_8O_6$), emodin ($C_{15}H_{10}O_5$), chrysophanol ($C_{15}H_{10}O_4$) and physcion ($C_{16}H_{12}O_5$) are calculated with reference to the dried drug.

Validate the methodology for accuracy, precision and repeatability, if necessary.

B.3 Determination of sennoside A

B.3.1 Preparation of test solution

Weigh accurately about 0,5 g of pulverized *Rheum* root and rhizome to a stoppered conical flask, add exactly 50 ml of a sodium hydrogen carbonate solution (1 in 1 000), shake for 30 min, filter and use the filtrate as the test solution.

B.3.2 Preparation of reference solution

Weigh accurately about 10 mg of sennoside A CRS, dissolve in a sodium hydrogen carbonate solution (1 in 1 000) to make exactly 50 ml. Pipette 5 ml of this solution and add sodium hydrogen carbonate solution (1 in 1 000) to make exactly 20 ml as the reference solution.

B.3.3 Chromatographic conditions

a) Stationary phase: octadecylsilane bonded silica gel.

b) Column size: 4,6 µm, 5 mm × 150 mm.

c) Mobile phase: a mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

d) Flow rate: 1 ml/min.

e) Injection volume: 10 µl.

f) Temperature of column oven: 30 °C.

g) Detector: a spectrophotometer set at 340 nm.

B.3.4 Determination

Accurately inject 10 µl of the test solution and reference solution, respectively, into the column and determine the peak areas, A_T and A_R, of sennoside A in each solution. HPLC chromatograms of sennoside A reference solution, *Rheum palmatum* root and rhizome sample, *Rheum tanguticum* root and rhizome sample and *Rheum officinale* root and rhizome are shown in Figure B.2. The mass fraction of sennoside A of the sample being examined on the dried basis, w_{ti} (%), is calculated with Formula (B.1):

$$w_{ti} = m_R/m_t \times A_T/A_R \times 1/4\,000 \times 100 \quad \text{(B.1)}$$

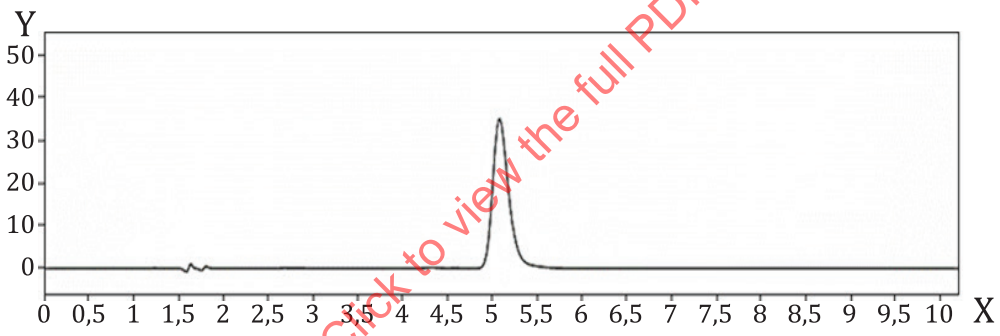
where

m_R is the mass of sennoside A CRS taken, in mg;

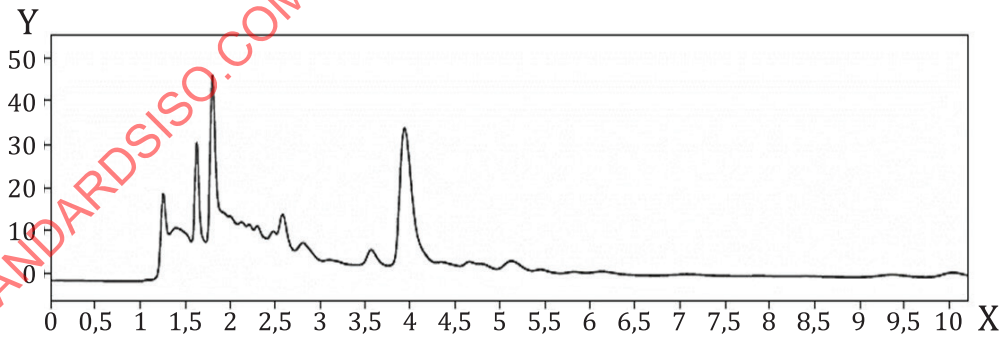
A_T is the peak area of sennoside A in the test solution;

A_R is the peak area of sennoside A in the reference solution;

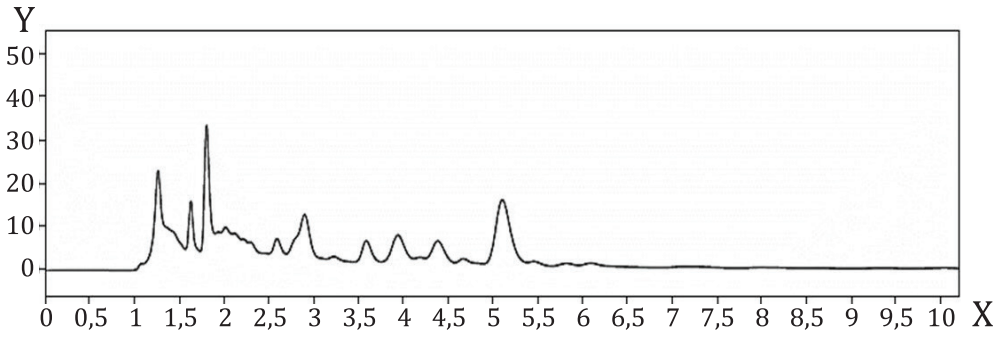
m_t is the mass of the test sample, in g.



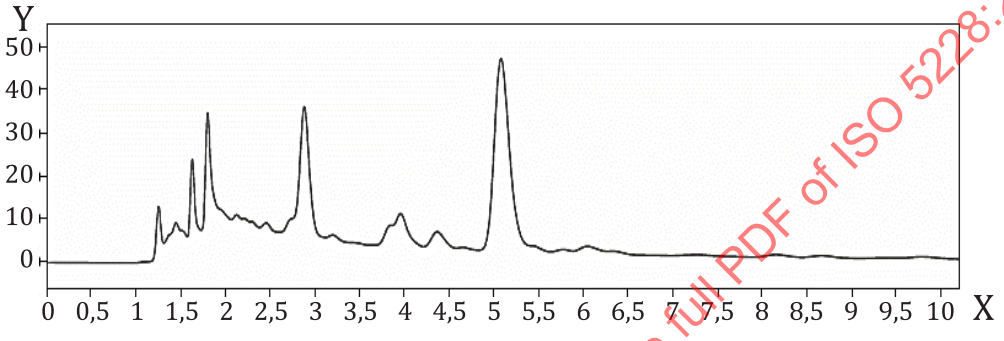
a) Sennoside A reference solution



b) *Rheum palmatum* root and rhizome sample



c) *Rheum officinale* root and rhizome sample



d) *Rheum tanguticum* root and rhizome sample

Key

X mAU

Y min

Figure B.2 — HPLC chromatograms of sennoside A determination

B.4 HPLC identification of rhaponticin

B.4.1 Preparation of test solution

Weigh accurately about 0,5 g of pulverized *Rheum* root and rhizome to a stoppered conical flask, add exactly 50 ml of methanol, ultrasonicate for 30 min, filter and use the filtrate as the test solution.

B.4.2 Preparation of reference solution

Weigh a certain amount of rhaponticin CRS accurately, dissolve with methanol and precisely dilute to 77 µg/ml as the reference solution.

B.4.3 Chromatographic conditions

a) Stationary phase: octadecylsilane bonded silica gel.

b) Column size: 4,6 µm, 5 mm × 150 mm.

c) Mobile phase A: a mixture of methanol and water (40:60).

d) Flow rate: 1 ml/min.

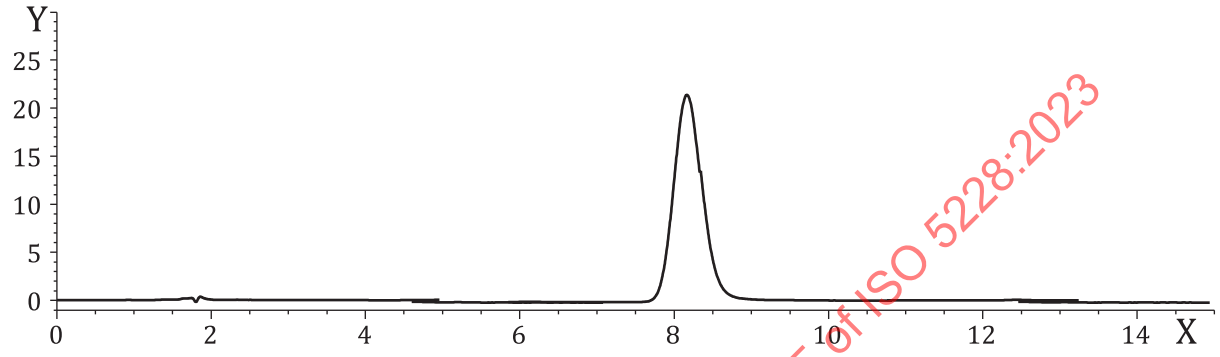
e) Injection volume: 10 µl.

f) Temperature of column oven: 30 °C.

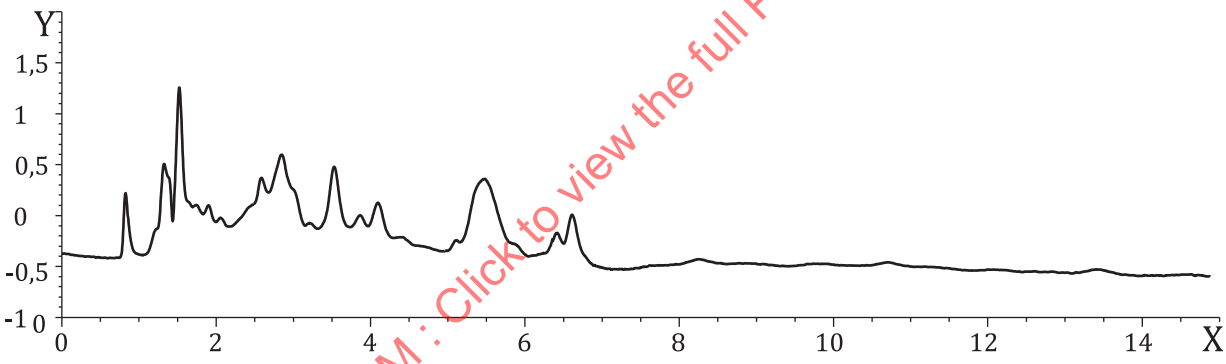
g) Detector: a spectrophotometer set at 320 nm.

B.4.4 Identification

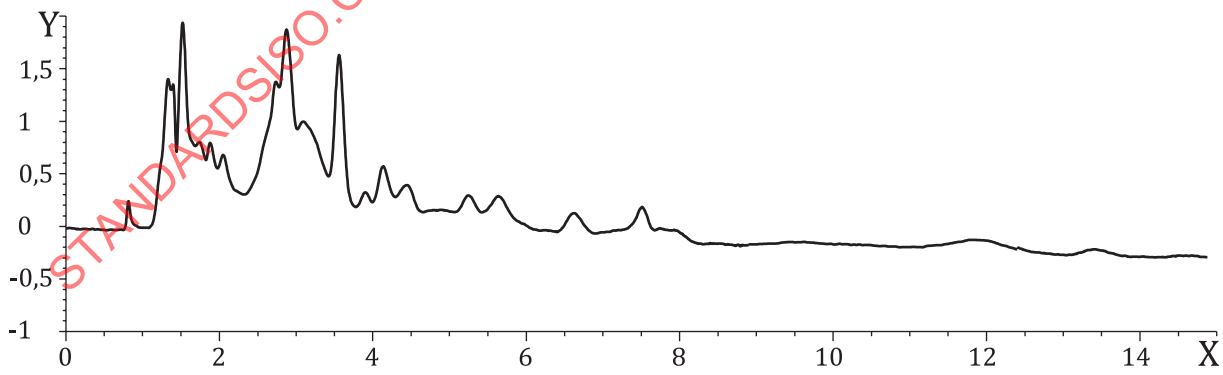
Accurately inject 10 µl each of the reference solution and the test solution into the column. HPLC chromatograms of rhaponticin reference solution, *Rheum palmatum* root and rhizome sample, *Rheum tanguticum* root and rhizome sample and *Rheum officinale* root and rhizome are shown in Figure B.3.



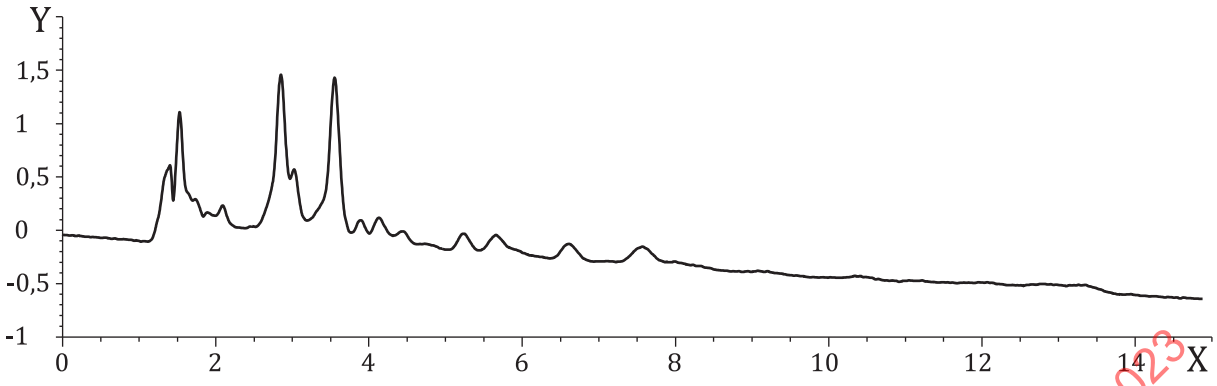
a) Rhaponticin reference solution



b) *Rheum palmatum* root and rhizome sample



c) *Rheum officinale* root and rhizome sample



d) *Rheum tanguticum* root and rhizome sample

Key

X mAU

Y min

Figure B.3 — HPLC chromatograms of rhaponticin identification