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Traditional Chinese medicine — *Salvia miltiorrhiza* root and rhizome

Médecine traditionnelle chinoise — Racine et rhizome de Salvia miltiorrhiza

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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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

Salvia miltiorrhiza Bge. is a perennial herbal plant in the Lamiaceae family. The root and rhizome of this plant have been used as traditional Chinese medicines for more than 2 500 years. Used as raw material, *Salvia miltiorrhiza* root and rhizome has been developed into various kinds of herbal medicine products and used in at least 19 countries or regions for their therapeutic value and market potential in the treatment of cardiovascular diseases, neurasthenic insomnia, liver fibrosis and cancer. It has also been used as an ingredient in health supplements and cosmetics.

Salvia miltiorrhiza Bge. is mainly grown and cultivated in China, but is also cultivated in the USA, Germany, Great Britain and Australia. However, the quality of *Salvia miltiorrhiza* root and rhizome provided by different cultivators is quite different, which may affect the safety and efficacy of this herb.

Salvia miltiorrhiza root and rhizome has been recorded by the United States of America Pharmacopoeia, the European Pharmacopoeia, the British Pharmacopoeia, the Chinese Pharmacopoeia, the Hong Kong Chinese Materia Medica Standard and the Taiwan Chinese Materia Medica Standard. However, these national or regional standards are not harmonized and may be unfavourable for the international trade of *Salvia miltiorrhiza* root and rhizome. One of the significant differences between them is that different chemical markers, or the same chemical markers with different limitations, are used in multiple standards. These chemical markers provide a vital index for judging the quality of *Salvia miltiorrhiza* root and rhizome. Therefore, the establishment of an international standard for *Salvia miltiorrhiza* root and rhizome is important for the international trade of this herb and also for ensuring the consistent quality and safety of this herb in clinical use.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.2.3, 5.2.4, 5.2.5, 5.2.6.1, 5.2.6.2, 5.2.7 and 5.2.8 in their national standards. Examples of national and regional values are given in Annex E.

Traditional Chinese medicine — *Salvia miltiorrhiza* root and rhizome

1 Scope

This document specifies the minimum requirements and test methods for *Salvia miltiorrhiza* root and rhizome, which is derived from the *Salvia miltiorrhiza* Bge plant.

It is applicable to *Salvia miltiorrhiza* root and rhizome that is sold and used as a natural medicine in international trade, including unprocessed and traditionally processed materials.

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 928, *Spices and condiments — Determination of total ash*

ISO 930, *Spices and condiments — Determination of acid-insoluble ash*

ISO 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO 20409, *Traditional Chinese medicine — Panax notoginseng root and rhizome*

ISO 21371, *Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use*

CODEX STAN 229-1993: *Analysis of pesticide residues: Recommended methods*

CAC/MRL01-2009: *Maximum Residue Limits for Pesticides in Foods*

World Health Organization. *Quality control methods for herbal materials: General advice on sampling*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

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

3.1
root and rhizome
underground part of *Salvia miltiorrhiza*

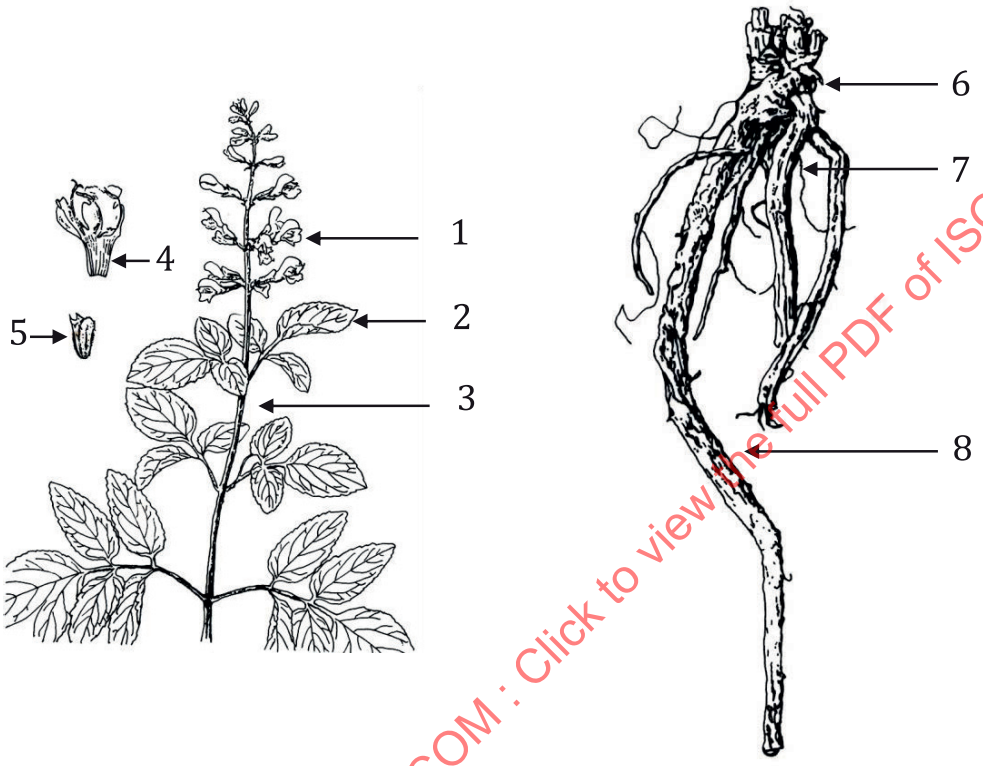
3.2
total ash
residue obtained after incineration at 525 ± 25 °C

3.3
acid-insoluble ash
part of the total ash remaining after treatment with hydrochloric acid

3.4
tanshinones
umbrella term of tanshinone IIA ($C_{19}H_{18}O_3$), cryptotanshinone ($C_{19}H_{20}O_3$) and tanshinone I ($C_{18}H_{12}O_3$)

4 Descriptions

Salvia miltiorrhiza root and rhizome is the dried root and rhizome of *Salvia miltiorrhiza* Bge. in the Lamiaceae (Labiatae) family as shown in Figure 1.



a) Aerial part of *Salvia miltiorrhiza* plant **b) Dried root and rhizome**

Key

1	raceme	5	calyx
2	leaf	6	rhizome
3	stem	7	fibrous root
4	flower (showing stamen)	8	root

Figure 1 — Structure of *Salvia miltiorrhiza*

5 Requirements

5.1 General characteristics

The following requirements shall be met before separating the bulk sample into test samples:

a) *Salvia miltiorrhiza* root and rhizome shall be clean and free from foreign matter.

b) 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 *Salvia miltiorrhiza* root and rhizome

5.2.1 Morphological features of root and rhizome

a) The rhizomes are short and stout, sometimes with a stem at the apex.

b) The roots are long, cylindrical and slightly curved. Some are branched and with rootlets.

c) The roots are 10 cm to 20 cm long, 0,3 cm to 1,5 cm in diameter.

d) The outer surface is brownish-red or dark brownish-red with rough and wrinkled longitudinal texture. The bark of old roots is loose, mostly purplish-brown and usually fall off.

e) The texture is hard and fragile.

f) The fracture is loose with cleft or slightly flat and compact with brownish-red cortex, greyish-yellow or purplish-brown xylem and yellowish-white xylem rays arranged radially.

5.2.2 Thin-layer chromatogram (TLC) identification

The identification of *Salvia miltiorrhiza* root and rhizome by thin-layer chromatogram (TLC) shall present spots or bands with a colour and position corresponding to those of reference solutions.

5.2.3 Moisture

The mass fraction of moisture should not be more than 13,0 %.

5.2.4 Total ash

The mass fraction of total ash should not be more than 10,0 %.

5.2.5 Acid insoluble ash limit

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

5.2.6 Extractives

5.2.6.1 Water-soluble extractives

The mass fraction of water-soluble extractives should not be less than 35,0 %.

5.2.6.2 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extractives should not be less than 15,0 %.

5.2.7 Content of tanshinones

The sum of mass fraction of tanshinone IIA ($C_{19}H_{18}O_3$), cryptotanshinone ($C_{19}H_{20}O_3$), and tanshinone I ($C_{18}H_{12}O_3$) should not be less than 0,2 %. Annex C provides further information for the method.

5.2.8 Content of salvianolic acid B

The mass fraction of salvianolic acid B ($C_{36}H_{30}O_{16}$) should not be less than 3,0 %. Annex D provides further information for the method.

5.2.9 Heavy metals

The content of heavy metals including arsenic, mercury, lead and cadmium shall be determined.

5.2.10 Pesticide residues

The content of pesticide residues such as Benzex, DDT (dichloro-diphenyl-trichloroethane) and quintozene shall be determined.

6 Sampling

Sampling of *Salvia miltiorrhiza* root and rhizome shall be in accordance with the World Health Organization's *Quality control methods for herbal materials: General advice on sampling*.

7 Test methods

7.1 Macroscopic identification

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

7.2 TLC identification

See Annex A for additional information.

7.3 Determination of moisture content

The testing method specified in ISO 20409 applies.

7.4 Determination of total ash

The testing method specified in ISO 928 applies.

7.5 Determination of acid-insoluble ash

The testing method specified in ISO 930 applies.

7.6 Determination of extractives

See Annex B for additional information.

7.7 Determination of tanshinones

See Annex C for additional information.

7.8 Determination of salvianolic acid B

See Annex D for additional information.

7.9 Determination of heavy metals

The testing method specified in ISO 18664 applies.

7.10 Determination of pesticide residues

The testing methods specified in CODEX STAN 229-1993 and CAC/MRL01-2009 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(s) used;

d) the test result(s) obtained;

e) all operational details not specified in this document, or regarded as optional, together with details of any incidents which may 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

Packaging 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 product shall be protected from light, moisture, pollution and entry of foreign substances during long-distance delivery.

10 Marking and labelling

Labelling requirements shall conform with ISO 21371.

a) product name and plant scientific name;

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

b) country and province/state where the products originated;

c) date of production, batch number and expiry date of the products;

d) storage and transportation method.

Annex A
(informative)

TLC identification

A.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 1 g of the powder, add 5 ml of ethanol, ultrasonicate for 15 min and take supernatant as the test solution.

A.2 Preparation of reference solutions

a) Weigh 1 g of *Salvia miltiorrhiza* root and rhizome reference drug powder and treat it in the same manner as in A.1 as the reference drug solution.

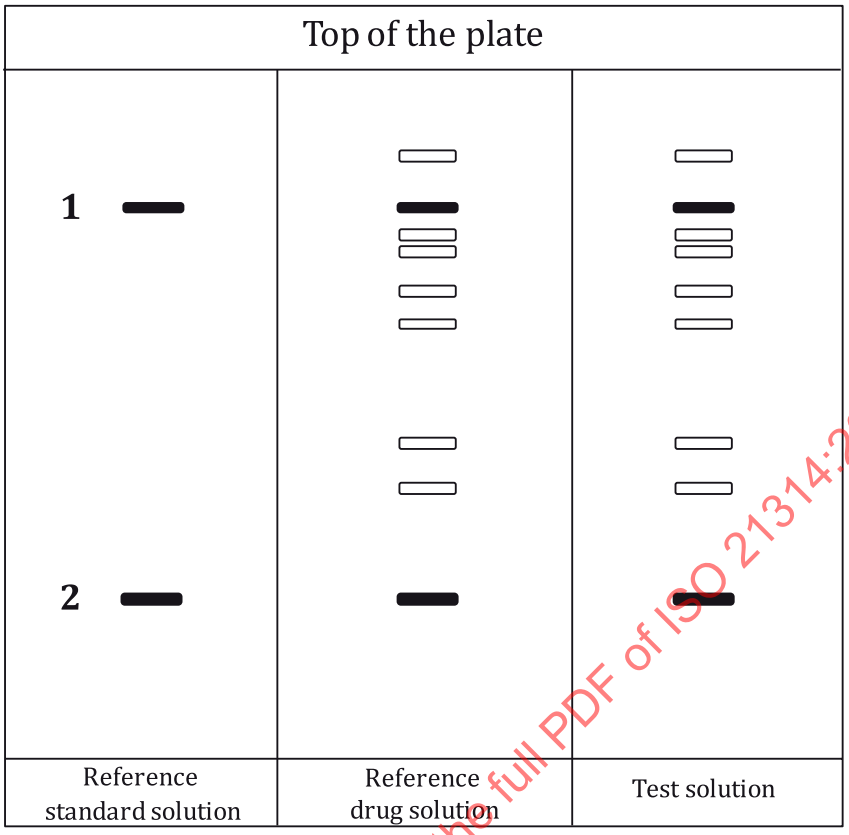
b) Dissolve tanshinone II_A CRS and salvianolic acid B CRS in ethanol to prepare a mixed reference standard solution (CRS or adequate qualities) containing 0,5 mg/ml of tanshinone II_A and 1,5 mg/ml of salvianolic acid B.

A.3 Developing solvent system

Prepare a mixture of trichloromethane, methylbenzene, ethyl acetate, methanol and formic acid in the volume ratio of 6:4:8:1:4 as the first mobile phase. Prepare a mixture of petroleum ether (60 approximately 90 °C) and ethyl acetate in the volume ratio of 4:1 as the second mobile phase.

A.4 Identification by TLC

Apply the reference standard solution, reference drug solution and test solution, each 5 µl, on the same TLC plate (silica gel) previously dried at 110 °C for 15 min in the oven. Develop the plate in the first mobile phase for a path of 4 cm, take the plate out and dry in air. Develop the plate in the second mobile phase for a path of 7,5 cm, take the plate out and dry in air. Examine the plate under both sunlight and ultraviolet light at 365 nm. Identify the spots of tanshinone II_A and salvianolic acid B and other spots of the test solution by comparing the positions and colours with these of the reference standard solution and reference drug solution. Typical reference TLC chromatograms are shown in Figure A.1.



Key

1 tanshinone II A

2 salvianolic acid B

Figure A.1 — Schematic diagram of typical TLC chromatograms of *Salvia miltiorrhiza* root and rhizome

Annex B
(informative)

Determination of extractives

B.1 Determination of water-soluble extractives

a) Weigh 250 g of sample to grind and pass it through a 24-mesh or coarse sieve. Dry the powder in a desiccator to a constant mass. Weigh approximately 4 g of the dried powder into a 250-ml stopper conical flask. Accurately add 100 ml of water.

b) Allow the mixture of the powder and water to stand at room temperature for 18 h. Stir the mixture from time to time during the first 6 h, then filter rapidly with a dry filter.

c) Transfer 25 ml of the successive filtrate into an evaporating dish. Evaporate the filtrate to dryness on a water bath.

d) Dry at 105 °C for 3 h and cool for 30 min in a desiccator. Weigh the extracts rapidly and accurately.

e) Calculate the mass fraction of water-soluble extractives, m_{wse}, on the dried basis (%) with Formula (B.1).

$$m_{wse} = (m_1 - m_0) \times 4 / m_s \times 100\,\% \qquad \text{(B.1)}$$

where

m_s is the mass of the sample (g);

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

m_0 is the mass of the evaporating dish (g).

B.2 Determination of ethanol-soluble extractives

a) Weigh 250 g of sample to grind and pass it through a 24-mesh or coarse sieve. Dry the powder in a desiccator to a constant mass. Weigh approximately 4 g of the dried powder into a 250-ml stopper conical flask. Accurately add 100 ml of 95 % ethanol and weigh.

b) Heat the mixture of the powder and ethanol stand under reflux to slightly boil on a water bath for 1 h. Cool and weigh again. Replenish the loss of mass with ethanol, mix well and filter.

c) Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into the evaporating dish. Evaporate the filtrate to dryness on a water bath.

d) Dry at 105 °C for 3 h and allow to cool for 30 min in a desiccator. Weigh the extracts rapidly and accurately.

e) Calculate the mass fraction of ethanol-soluble extractives on the dried basis (%) with Formula (B.1).

Annex C
(informative)

Determination of tanshinones

C.1 Principle of the test method

The high performance liquid chromatography (HPLC) method is employed to determine the content of tanshinones. The HPLC system consists of a quaternary pump, a continuous vacuum degasser, a thermostated auto-sampler and a column compartment coupled to a variable wavelength diode-array detector.

C.2 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Accurately weigh 0,3 g of the powder in a 250-ml stopper conical flask. Accurately add 50 ml of methanol. Ultrasonic treatment (powder: 140 W, rate: 42 kHz) for 30 min. Cool and weigh again. Replenish the loss of mass with methanol and mix well. Filter and use the successive filtrate. Filter through a 0,45 µm membrane filter as the test solution.

C.3 Preparation of reference standard solution

Accurately weigh a quantity of tanshinone IIA CRS to an amber measuring flask, dissolve in methanol to prepare a solution containing 20 µg per ml as the reference solution.

C.4 Chromatographic system and HPLC assay

C.4.1 Column.

a) Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

b) Size: l = 0,25 m, $\varnothing$ = 4,6 mm, particle size = 5,0 µm.

c) Theoretical plates: not less than 60 000.

C.4.2 Mobile phase.

a) Mobile phase A: acetonitrile.

b) Mobile phase B: 0,02 % phosphoric acid solution.

c) Program of gradient elution.

Time (min)	Mobile phase A (% volume fraction)	Mobile phase B (% volume fraction)
0 to 6	61	39
6 to 20	61 to 90	39 to 10
20 to 20,5	90 to 61	10 to 39
20,5 to 25	61	39

C.4.3 Flow rate: 1 ml/min.

C.4.4 Detector: 270 nm.

C.4.5 Bge.: 20 °C.

C.4.6 Injection volume: 10 µl.

C.4.7 Calculation conversion: calculate the relative retention time of both cryptotanshinone and tanshinones I by comparing with reference tanshinone IIA, whose corresponding peak is S-peak. The value should be within ± 5 % of the specified value. Table C.1 shows relative retention time and correction factor.

Table C.1 — Relative retention time and correction factor

Component to be tested (peak)	Relative retention time	Correction factor
Cryptotanshinone	0,75 (0,71 approximately 0,79)	1,18
Tanshinone I	0,79 (0,75 approximately 0,83)	1,31
Tanshinone IIA	1,00	1,00

C.5 Content calculation of tanshinones

C.5.1 The amount of tanshinone IIA is calculated with Formula (C.1):

$$m_{\mathrm{tIIA}} = \frac{A_{\mathrm{t}} \times C_{\mathrm{r}} \times P \times 10^{-6} \times 50}{A_{\mathrm{r}}} \tag{C.1}$$

where

m_{tIIA} is the mass of tanshinone IIA in the sample (g);

A_{t} is the area of the peak due to tanshinone IIA ($C_{19}H_{18}O_3$) in the chromatogram obtained with the test solution;

C_{r} is the content of tanshinone IIA CRS in the reference solution (g/ml);

P is the percentage content of tanshinone IIA ($C_{19}H_{18}O_3$)in tanshinone IIA CRS;

A_{r} is the area of the peak due to tanshinone IIA ($C_{19}H_{18}O_3$) in the chromatogram obtained with the reference solution.

C.5.2 The amount of cryptotanshinone is calculated with Formula (C.2):

$$m_{\mathrm{c}} = \frac{A_{\mathrm{c}} \times C_{\mathrm{r}} \times P \times 10^{-6} \times 50}{A_{\mathrm{r}}} \times 1{,}18 \tag{C.2}$$

where

m_{c} is the mass of cryptotanshinone in the sample (g);

A_{c} is the area of the peak due to cryptotanshinone ($C_{19}H_{20}O_3$) in the chromatogram obtained with the test solution;

C_{r}, P and A_{r} are the same as in Formula (C.1).

C.5.3 The amount of tanshinone I is calculated with Formula (C.3):

$$m_{\mathrm{tI}} = \frac{A_{\mathrm{t}} \times C_{\mathrm{r}} \times P \times 10^{-6} \times 50}{A_{\mathrm{r}}} \times 1{,}31 \qquad \text{(C.3)}$$

where

m_{tI} is the mass of tanshinone I in the sample (g);

A_{t} is the area of the peak due to tanshinone I ($C_{18}H_{12}O_3$) in the chromatogram obtained with the test solution;

C_{r}, P and A_{r} are the same as in Formula (C.1).

C.5.4 The content of tanshinones is calculated with Formula (C.4):

$$\frac{m_{\mathrm{tIIA}} + m_{\mathrm{c}} + m_{\mathrm{tI}}}{m_{\mathrm{s}} \times (1 - C_{\mathrm{m}})} \times 100\,\% \qquad \text{(C.4)}$$

where

m_{tIIA} is the same as in Formula (C.1);

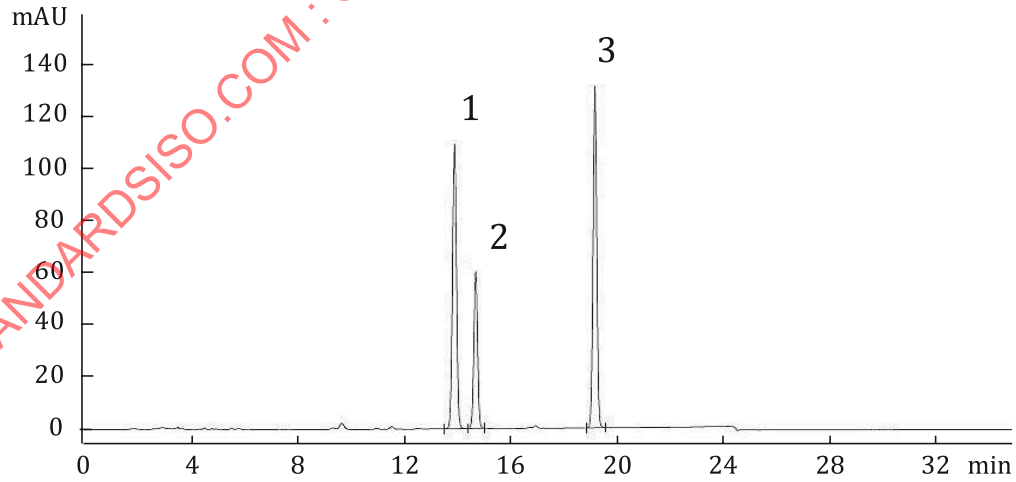
m_{c} is the same as in Formula (C.2);

m_{tI} is the same as in Formula (C.3);

m_{s} 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.

C.5.5 A typical reference HPLC chromatogram is shown in Figure C.1.



Key

1 cryptotanshinone
2 tanshinone I
3 tanshinone IIA

Figure C.1 — Typical reference HPLC chromatogram of tanshinones

Annex D
(informative)

Determination of salvianolic acid B

D.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80-mesh or finer sieve. Accurately weigh 0,15 g of the powder in a 250-ml stopper conical flask. Accurately add 50 ml of a mixture of methanol and water (8:2). Weigh and use ultrasonic treatment (powder: 140 W, rate: 42 kHz) for 30 min. Cool and weigh again. Replenish the loss of mass with a mixture of methanol and water (8:2), mix well and filter. Then accurately weigh 5 ml successive filtrate in a 10 ml volumetric flask and add a mixture of methanol and water (8:2). Dilute the solution to 10 ml. Filter through a 0,45 µm membrane filter as the test solution.

D.2 Preparation of reference standard solution

Accurately weigh a quantity of salvianolic acid B CRS to a measuring flask, then dissolve in a mixture of methanol and water (8:2) to prepare a solution containing 0,1 mg/ml as the reference standard solution.

D.3 Chromatographic system and HPLC assay

D.3.1 Column.

a) Stationary phase: octadecylsilane bonded silica gel as analysing column or equivalent.

b) Size: l = 0,25 m, $Ø$ = 4,6 mm, particle size = 5,0 µm.

c) Theoretical plates: not less than 6 000.

D.3.2 Mobile phase: a mixture of acetonitrile, 0,1 % phosphoric acid solution (22:78).

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

D.3.4 Bge.: 20 °C.

D.3.5 Detector: 286 nm.

D.3.6 Injection volume: 10 µl.

D.4 Content calculation of salvianolic acid B

D.4.1 The content of salvianolic acid B is calculated with Formula (D.1):

$$\frac{A_1 \times C_r \times P \times 10^{-3} \times 100}{A_2 \times m \times (1 - C_m)} \times 100\,\% \qquad \text{(D.1)}$$

where

A_1 is the area of the peak due to salvianolic acid B ($C_{36}H_{30}O_{16}$) in the chromatogram obtained with the test solution;

A_2 is the area of the peak due to salvianolic acid B ($C_{36}H_{30}O_{16}$) in the chromatogram obtained with the reference solution;

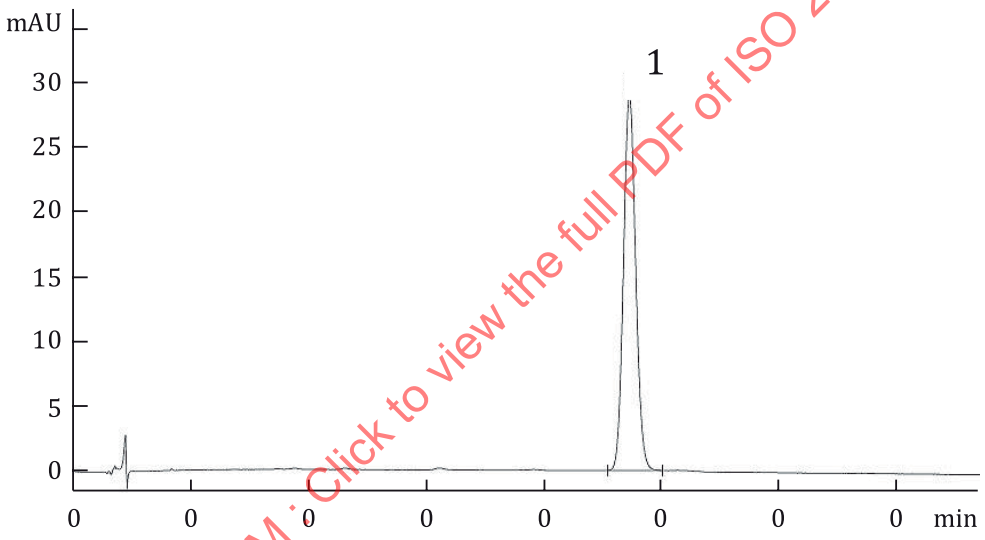
C_r is the content of ferulic acic CRS in the reference solution (g);

P is the percentage content of salvianolic acid B ($C_{36}H_{30}O_{16}$) in salvianolic acid CRS;

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.

D.4.2 A typical reference HPLC chromatogram is shown in Figure D.1.



Key

1 salvianolic acid

Figure D.1 — Typical reference HPLC chromatogram of salvianolic acid B