INTERNATIONAL STANDARD

ISO 20409

First edition
2017-06

Traditional Chinese medicine — *Panax notoginseng* root and rhizome

Médecine traditionnelle chinoise — Rhizome et racine de Panax notoginseng

Reference number
ISO 20409:2017(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Introduction

Panax notoginseng root and rhizome are medicinal parts of *Panax notoginseng* (Burk.) F.H. Chen named *Sanqi*, which is a well-known traditional Chinese medicine. Due to its ability to treat bleeding, blood stasis and some other blood disorders, *Panax notoginseng* root and rhizome soared to great importance during Qing dynasty times after being praised by the master herbalist Li Shizhen in the 16th century. *Panax notoginseng* root and rhizome are native to the southern Chinese provinces of Yunnan and Guangxi, as well as Vietnam. The root and rhizome exhibit a variety of botanical and biochemical similarities to ginseng, and are frequently consumed as soup. Despite this high rate of consumption, there are relatively few reported side effects, making *Panax notoginseng* root and rhizome two of the safest substances in traditional Chinese medicine. While most often consumed as a popular food tonic, practitioners of Oriental medicine know *Panax notoginseng* root and rhizome best for the medicinal qualities: moving stagnant blood, stopping bleeding and resolving swelling. Due to the high price and demand in the global market, trade in *Panax notoginseng* root and rhizome has been complicated by substitution, adulteration and species identification issues. The genuine material is often replaced by less valuable material(s), some of which exhibit potentially toxic properties. Therefore, the establishment of an international standard for *Panax notoginseng* root and rhizome is necessary to support the clinical effectiveness, safety and consistency of this valuable medicine in international trade.

Traditional Chinese medicine — *Panax notoginseng* root and rhizome

1 Scope

This document specifies minimum requirements and test methods for notoginseng root and rhizome which are derived from the plant *Panax notoginseng* (Burk.) F.H. Chen.

It is applicable to notoginseng root and rhizome that are sold and used as food supplements, functional food or natural medicines 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 1575, *Tea — Determination of total ash*

ISO 1577, *Tea — Determination of acid-insoluble ash*

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

CODEX STAN 229-1993, REV.1-2003: *Analysis of pesticide residues: Recommended methods*

CODEX STAN 1-1985: *Codex general standard for the labelling of prepackaged foods*

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

World Health Organization 2011: *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:

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

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

3.1
notoginseng
plant of *Panax notoginseng* (Burk.) F. H. Chen that has been cultivated for at least three years

3.2
root weight
average weight of final samples of root

3.3
root length
largest distance from the bottom to the stem scar of the tap root

Note 1 to entry: See Figure 1.

3.4
root diameter
diameter of the tap root

3.5
root number per 500 g
number of tap roots per 500 g

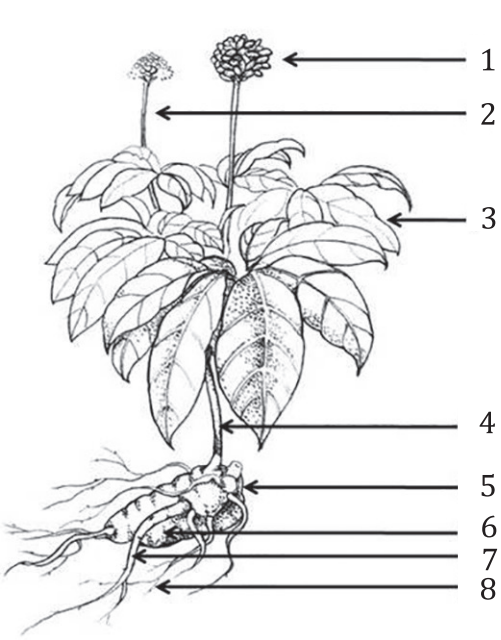
3.6
batch
samples collected from the same particular place at the same time

Note 1 to entry: This is not more than 5 000 kg.

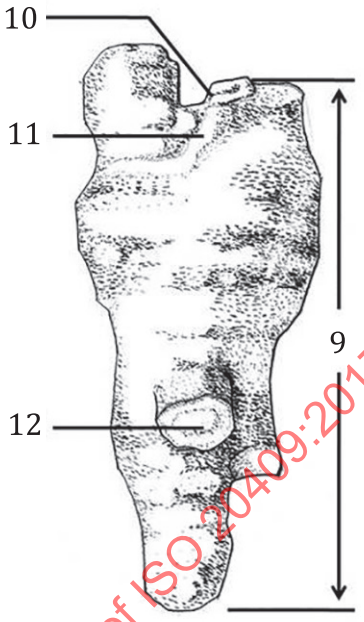
3.7
final sample
samples after the final sampling

4 Descriptions

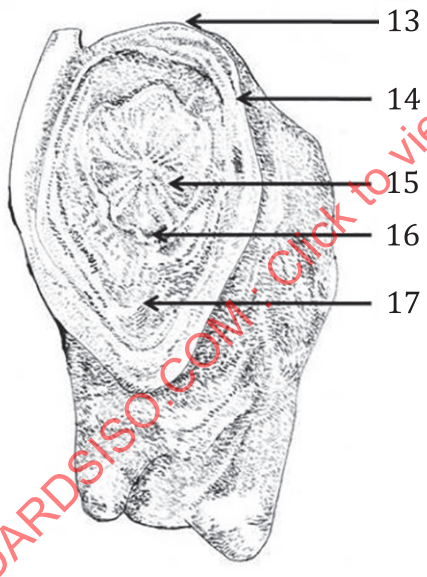
In this document, dried notoginseng root and rhizome consist of tap root, lateral root and rhizome, as shown in Figure 1.



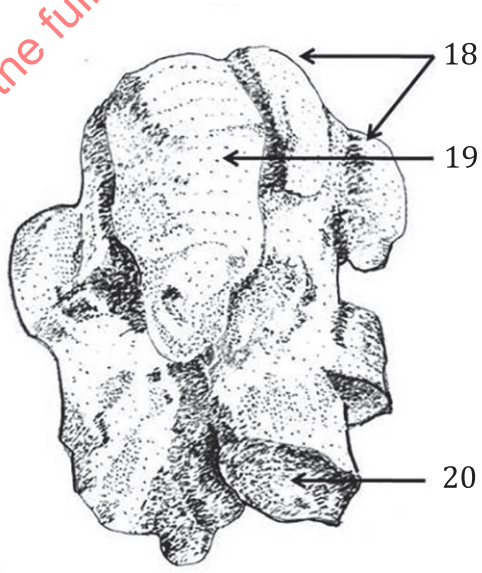
a) Plant of *Panax notoginseng*



b) Dried tap root



c) Transverse section of the tap root



d) Dried rhizome

Key

1	fruit	8	rootlets	15	ray
2	flower	9	tap root length	16	xylem
3	leaf	10	stem scar	17	central cylinder
4	stem	11	warty protrusion	18	stem scar
5	rhizome	12	lateral root scar	19	strip-shaped protruding
6	tap root	13	epidermis	20	root scar
7	lateral root	14	phloem		

Figure 1 — Structure of notoginseng

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling.

a) Notoginseng root and rhizome shall be clean and free from rootlets and 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 Notoginseng root

5.2.1 Morphological features of root

a) The shape of the tap root is subconical or cylindrical as shown in Figure 1 b).

b) The root length is 1 cm to 6 cm long, and the root diameter is 1 cm to 4 cm.

c) The outer surface is greyish-yellow or greyish-brown with intermittent longitudinal wrinkles and branch root scar.

d) Stem scars at the apex are surrounded by warty protrusions.

e) The texture is heavy and compact.

f) The fracture is greyish-green, yellowish-green or greyish-white with xylem rays distributed radially.

5.2.2 Moisture

The mass fraction of moisture shall not be more than 12,0 % (w/w).

5.2.3 Total ash

The mass fraction of total ash shall not be more than 6,0 % (w/w).

5.2.4 Acid-insoluble ash

The mass fraction of acid-insoluble ash shall not be more than 1,0 % (w/w).

5.2.5 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extractives shall be more than 16,0 % (w/w).

5.2.6 Identification of notoginsenoside R_1 and ginsenosides Rg_1, Re, and Rb_1

The identification of notoginsenoside R_1 and ginsenosides Rg_1, Re, and Rb_1 with thin-layer chromatogram (TLC) or high-performance liquid chromatogram (HPLC) shall present spots or peaks obtained from the test and reference solutions in the same position with the same colour (TLC) or same absorbance curve (HPLC).

5.2.7 Content of notoginsenoside R_1 and ginsenosides Rg_1 and Rb_1

The sum of the mass fraction of notoginsenoside R_1 and ginsenosides Rg_1 and Rb_1 shall be not less than 5,0 %. The content determination is carried out according to the method described in Annex B.

5.2.8 Heavy metals

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

5.2.9 Pesticide residues

The contents of pesticide residues including Benzex, DDT and quintozene shall be determined.

5.2.10 Root weight, root length and root number per 500 g

The root weight, root length and root number per 500 g of each batch of notoginseng root shall comply with the requirements in Table 1. The quantity of roots that fail to meet the minimum weight requirement of the grade shall not be more than 5 %. Otherwise, it shall be considered to be of inferior grade.

Table 1 — Grading requirements of notoginseng root

Grade	Root weight g	Root length cm	Root number per 500 g
First	≥25,0	≤6,5	≤20
Second	≥17,0	≤6,0	≤30
Third	≥12,5	≤5,5	≤40
Fourth	≥8,5	≤4,5	≤60
Fifth	≥6,5	≤3,5	≤80
Sixth	≥4,5	≤3,0	≤120
Seventh	≥2,5	≤2,5	≤200
Unqualified	<2,5	>2,5	>200
NOTE 1 The root weight is determined when the moisture content of the tap root is approximately 12 %. NOTE 2 The grading requirements are established according to the traditional grading system of notoginseng root and rhizome that has long been extensively used in the market and trading. The grade shall be established only when all three requirements, i.e. root weight, root length, and root number per 500 g, are met.			

5.3 Notoginseng rhizome

5.3.1 Morphological features of rhizome

The appearance of rhizome is irregularly shrunken, lump-shaped or slat-shaped, and there are several conspicuous stem scars and annulations on the surface of rhizome as shown in Figure 1 d). The fracture is greyish-green, or greyish-white in the centre and deep green or grey at margin.

5.3.2 Moisture

The mass fraction of moisture shall not be more than 14,0 % (w/w).

5.3.3 Total ash

The mass fraction of total ash shall not be more than 6,0 % (w/w).

5.3.4 Acid-insoluble ash

The mass fraction of acid-insoluble ash shall not be more than 3,0 % (w/w).

5.3.5 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extractives shall be more than 20,0 % (w/w).

5.3.6 Identification of notoginsenoside R_1 and ginsenosides Rg_1, Re, and Rb_1

The identification of notoginsenoside R_1 and ginsenosides Rg_1, Re, and Rb_1 with TLC or HPLC shall present spots or peaks obtained from the test and reference solutions in the same position with the same colour (TLC) or same absorbance curve (HPLC).

5.3.7 Content of notoginsenoside R_1 and ginsenosides Rg_1 and Rb_1

The sum of the mass fraction of notoginsenoside R_1 and ginsenosides Rg_1 and Rb_1 shall be not less than 8,0 %. The content determination is carried out according to the method described in Annex B.

5.3.8 Heavy metals

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

5.3.9 Pesticide residues

The contents of pesticide residues including Benzex, DDT and quintozene shall be determined.

6 Sampling

Sampling of notoginseng root or rhizome shall be done with reference to the World Health Organization 2011: *Quality Control Methods for Herbal Materials* (Updated edition of quality control methods for medicinal plant materials, 1998), *General Advice on Sampling*.

a) From a batch of five containers or packaging units, take a sample from each one.

b) From a batch of 6 to 50 units, take a sample from five.

c) From a batch of over 50 units, sample 10 %, rounding up the number of units to the nearest multiple of ten. For example, a batch of 51 units would be sampled as for 60, i.e. take samples from six packages.

d) From each container or package selected, take three original samples from the top, middle and bottom of the container or package. The three original samples should then be combined into a pooled sample that should be mixed carefully.

e) The average sample is obtained by quartering. From the pooled sample, adequately mix into an even and square-shaped heap, and divide it diagonally into four equal parts. Take two diagonally opposite parts and mix carefully.

f) Repeat the process as necessary until the required quantity, to within ±10 %, is obtained.

g) Using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material.

h) The final samples are tested for the measurement and analyses specified in Table 2.

Table 2 — Maximum weight of batch and minimum weight of the final sample

Maximum weight of root and rhizome batch kg	Minimum weight of final sample g		
	For measure of root weight, root length and root number per 500 g	For analysis of notoginsenoside R1 and ginsenosides Rg1, Re and Rb1	For other analyses
5 000	500	250	250
NOTE 1 The requirements are based on roots and rhizomes collected from different production regions of notoginseng. NOTE 2 Other analyses include the identification and the determination of moisture content, root weight, total ash, acid-insoluble ash, ethanol-soluble extractives, heavy metals and pesticide residues.			

7 Test methods

7.1 Macroscopic identification

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

7.2 Determination of moisture content

The testing method specified in Annex A applies.

7.3 Determination of total ash content

The testing method specified in ISO 1575 applies.

7.4 Determination of acid-insoluble ash content

The testing method specified in ISO 1577 applies.

7.5 Determination of ethanol-soluble extractives content

The testing method specified in Annex B applies.

7.6 Identification of notoginsenoside R_1 and ginsenosides Rg_1, Re, and Rb_1

The testing method specified in Annex C applies.

7.7 Determination of notoginsenoside and ginsenosides content

The testing method specified in Annex D applies.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 applies.

7.9 Determination of pesticide residues

The testing methods specified in CODEX STAN 229-1993 and CAC/MRL01-2009 apply.

7.10 Root weight

Samples of not less than 500 g are taken from each batch randomly. The roots are weighed one by one. The average weight of samples is then calculated.

7.11 Root length

Samples of not less than 500 g are taken from each batch randomly. The length from the bottom to the stem scar of the tap root is measured one by one. The average length of samples is then calculated.

7.12 Root number per 500 g

Samples of not less than 500 g are taken from each batch randomly. The samples are weighed together accurately to 0,1 g and counted. The number of roots per 500 g is calculated by using Formula (1):

$$\text{root number/500 g} = (500\ \text{g} \times \text{number of samples})/\text{weight of samples} \quad (1)$$

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 20409:2017;

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 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

The packaging shall not transmit any odour or flavour to the product and shall not contain substances that may damage the product or constitute a health risk.

The temperature for notoginseng storage shall not be higher than 25 °C. Notoginseng storage time shall not exceed 36 months.

10 Marking and labelling

Refer to the method specified in the CODEX STAN 1-1985. The following items shall be marked or labelled on the packages:

a) grade of the product in accordance with 5.2.8;

b) all quality features indicated in 5.2 and 5.3, determined in accordance with methods specified in Clause 7;

c) maximum weight of the batch and the minimum weight of samples specified in Table 2;

d) country and province/state of origin of the products;

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

f) storage method.

Annex A
(normative)

Determination of moisture content

Moisture content in notoginseng can be determined by the oven drying method.

Determination can be conducted in terms of the following steps.

a) Weigh 2 g to 5 g of sample powder and lay on the dried flat weighing bottle, the thickness of which is not more than 5 mm. The thickness of the sample powder is not more than 10 mm. Accurately weigh the bottle after.

b) Dry the bottle at 100°C to 105°C for 5 h with the bottle cap opened. Cover the cap and transfer the bottle into the dryer to cool for 30 min. Accurately weigh the bottle after.

c) Dry the bottle at the above temperature for 1 h. Cool the bottle and weigh it, until the weight difference of two successive weighing is not more than 5 mg.

d) According to the weight loss, calculate the moisture content of samples (%) with Formula (A.1):

$$\text{Moisture content (\%)} = (W0 - W1) / W0 \times 100\ \% \quad \text{(A.1)}$$

where

$W0$ is the weight of the flat weighing bottle and samples before drying (g);

$W1$ is the weight of the flat weighing bottle and samples after drying (g).

Annex B
(normative)

Determination of ethanol-soluble extractives content

Ethanol-soluble extractives content in notoginseng can be determined by the hot-dip method.

Determination can be conducted in terms of the following steps.

a) Weigh 250 g of the sample to grind and pass it through a sieve of 24 mesh or a coarse sieve. Weigh approximately 0,5 g of the powder into a 250 ml stopper conical flask. Accurately add 50 ml ethanol. Weigh and allow to stand for 1 h.

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

c) Weigh a dried evaporating dish. 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 allow to cool for 30 min in a desiccator. Weigh the extracts rapidly and accurately.

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

$$\text{Ethanol-soluble extractives (\%)} = (W1 - W0) \times 2 / S \times 100 \quad \text{(B.1)}$$

where

S is the weight of the sample (g);

$W1$ is the weight of the evaporating dish and residue after drying (g);

$W0$ is the weight of the evaporating dish (g).

Annex C
(normative)

Identification of ginsenosides Rb_1, Re, Rg_1 and notoginsenoside R_1

C.1 Preparation of the test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Weigh approximately 0,5 g of the powder into a 250 ml stopper conical flask, add five drops of water and mix well. Add 5 ml of water and saturated 1-butanol into the flask and stop it closely. Shake for 10 min and then allow to stand for 2 h. Centrifuge and then take the supernatant for mixing with 3 × volume of 1-butanol saturated water. Mix well and allow to stand until separation into two layers occurs. Take the layer of 1-butanol and evaporate to dryness. Dissolve the residue with 1 ml of methanol as the test solution.

C.2 Preparation of reference standards solution

Dissolve reference standards of ginsenosides Rb_1, Re, Rg_1 and notoginsenoside R_1 in methanol to prepare the reference standards solution of 0,5 mg/ml.

C.3 Identification by TLC

Apply 1 µl of the reference standard solution and 1 µl of test solution on identical TLC plates (silica gel) previously dried at 110 °C for 15 min in the oven. Develop with a solution of the mixture of chloroform, ethylacetate, methanol and water (15:40:22.10, v/v/v/v) below 10 °C. Take the plate out and dry in air. Spray 10 % sulfuric acid or 30 % sulfuric acid-ethanol solution over the TLC plate and heat at 105 °C until the colour looks clear. Identify the notoginsenoside and ginsenosides spots of test solution by comparing the positions and colours with those of the reference standard solution. Typical reference TLC chromatograms are shown in Figure C.1.

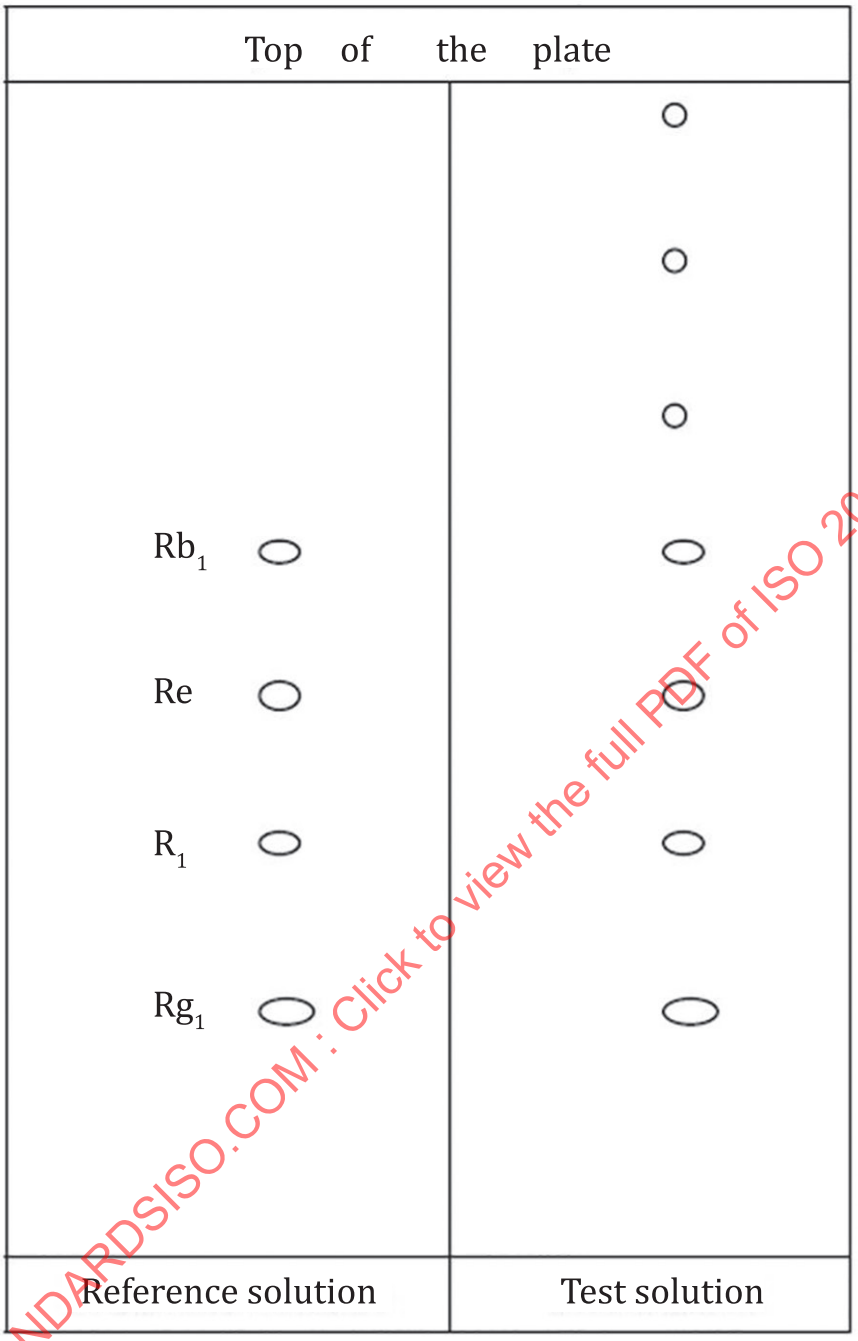


Figure C.1 — Schematic diagram of typical reference TLC chromatograms of notoginseng

Annex D
(normative)

Determination of notoginsenoside R_1 and ginsenosides Rg_1 and Rb_1

D.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Weigh accurately 0,6 g of the powder in a 250 ml stopper conical flask. Accurately add 50 ml methanol. Weigh and allow to stand overnight. Heat it under reflux to slightly boil on a water bath at 80 °C for 2 h. Cool and weigh again. Replenish the loss of solvent with methanol and mix well. Filter and use the successive filtrate. Filter through 0,45 µm membrane filter as the test solution.

D.2 Preparation of reference standards solution

Dissolve reference standards, i.e. notoginsenoside R_1 and ginsenoside Rg_1 and Rb_1 with methanol to make a mixture of 0,1 mg/ml, 0,4 mg/ml, 0,4 mg/ml as the reference standards solution.

D.3 Chromatographic system and HPLC assay

D.3.1 Column

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

D.3.1.2 Size: l = 0,25 m, $\varnothing$ = 4,6 mm

D.3.2 Mobile phase

D.3.2.1 Mobile phase A: acetonitrile

D.3.2.2 Mobile phase B: water for chromatography

D.3.2.3 Program of gradient elution

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 to 20	20	80
20 to 45	20→46	80→54
45 to 55	46→55	54→45
55 to 60	55	45

D.3.3 Flow rate: 1 ml/min

D.3.4 Detector: 203 nm

D.3.5 Injection volume: 10 µl