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Traditional Chinese medicine — *Astragalus mongholicus* root

*Médecine traditionnelle chinoise — Racine d'*astragalus mongholicus

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The *Astragalus* root (also called *Astragali Radix*, Milkvetch Root, Huang Qi in Mandarin, or 黄芪 or 黄耆 in Han characters) is one of the most frequently used herbal medicines in traditional Chinese medicine (TCM).

Of roughly 80 000 TCM formulae recorded in classic TCM books, around 7,3 % contain the *Astragalus* root as an ingredient. For example, of 1 493 formulae in the Chinese Pharmacopeia (2015 edition), there are 202 which contain the *Astragalus* root (accounting for 13,53 %); among 148 kampo medicines for prescription from the Ministry of Health, Labour and Welfare (MHLW) of Japan, there are 15 formulae containing the *Astragalus* root (accounting for 10,14 %). In the United States, the *Astragalus* root is widely sold as a dietary supplement ingredient.

Based on the statistics report for the Department of Market Supervision, Ministry of Commerce of the People's Republic of China, the volume of *Astragalus* exported from China in 2015 was 4 477,2 tons, worth 28,330,614 US$. The destination countries and regions included Malaysia, the United States, Japan, South Korea, Australia, Thailand, Singapore, Indonesia, Vietnam, Hong Kong and Taiwan.

The *Astragalus* root has been used in TCM for a very long time and remains a highly valued herb today because of its significant effects, which include:

— Efficacy supported by modern research: controls inflammation, boosts the immune system, slows or prevents the growth of tumours, protects the cardiovascular system, regulates and prevents diabetes and comorbidities related to diabetes, contains antioxidative and anti-ageing capabilities, heals wounds, alleviates symptoms of chemotherapy, treats cold and flu, and provides supplemental therapy for chronic asthma.

— Traditional indications: qi deficiency and lack of strength, reduced food intake, sloppy stool, sunken middle qi, chronic diarrhoea, prolapse of the rectum, bloody stool, flooding and spotting, exterior deficiency with spontaneous sweating, qi deficiency oedema, interior heat wasting-thirst, blood deficiency and sallow complexion, hemiplegia, impediment pain, numbness, abscesses and long-term nonhealed cellulitis caused by diabetic complications.

Two species of *Astragalus*, namely *Astragalus mongholicus* and *Astragalus membranaceus*, are included in the British Pharmacopoeia[1], the Pharmacopoeia of the People's Republic of China[2], the European Pharmacopoeia[3], the Japanese Pharmacopoeia[4], the Korean Pharmacopoeia[5] and the United States Pharmacopoeia[6] (see Table F.1). This document deals with *Astragalus mongholicus*; while ISO/NP 21311 deals with *Astragalus membranaceus*. These two species are different in terms of morphology and identification. *Astragalus membranaceus* has small numbers of large leaflets, while *Astragalus mongholicus* has large numbers of small leaflets. These species can be identified and differentiated by the HPLC method[7] and DNA barcoding[8]. There are also many other *Astragalus* species that are morphologically similar to *Astragalus mongholicus*, some of which are toxic. The toxic species of *Astragalus* include *Astragalus emoryanus* var. *emoryanus*, *Astragalus emoryanus* var. *terlinguensis*, *Astragalus miser* var. *oblongifolius*, *Astragalus miser* var. *serotinus*, *Astragalus miser* var. *hylophilus*, *Astragalus michauxii*, *Astragalus Canadensis*, *Astragalus oreganus*, *Astragalus variabilis*, *Astragalus strictus*, *Astragalus hamiensis*, *Astragalus tibetanus*, *Astragalus confertus*, *Astragalus rigidulus*, and *Astragalus leucoephalus*, which can contain poisonous substances such as miserotoxin, karakin, cibarin and hiptugin[9]. Thus, the establishment of an ISO standard for *Astragalus mongholicus* root is necessary to ensure the quality and safe use of this herb.

The misuse of such species in TCM remains a problem resulting in questions about the effectiveness or unexpected side-effects of *Astragalus mongholicus* and *Astragalus membranaceus*. The establishment of an international standard for *Astragalus mongholicus* root is therefore necessary to guarantee the clinical effectiveness, safety and controllability of this valuable medicine in global commerce and trade. Reference information on the use of *Astragalus* root in different countries and regions is included in Annex F.

As national implementation may differ, national standards bodies are invited to modify the values given in 5.2, 5.3 and 5.4 in their national standards. Examples of national and regional values are given in Annex F.

Traditional Chinese medicine — Astragalus mongholicus root

1 Scope

This document specifies the quality and safety requirements of *Astragalus mongholicus* root [root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao].

This document applies to *Astragalus mongholicus* root that is sold and used as natural medicine in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces derived from this plant.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1575, *Tea — Determination of total ash*

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,[1)] *Traditional Chinese medicine — Storage requirements for raw materials and decoction pieces*

ISO 22258,[2)] *Traditional Chinese medicine — Determination of pesticide residues in natural products by GC*

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
***Astragalus mongholicus* root**
dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao

Note 1 to entry: For synonyms of the *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, see Table F.1.

3.2
root length
distance from the bottom to the stem scar of the tap root, in centimetres

1) Under preparation. Stage at the time of publication: ISO/DIS 22217:2019.

2) Under preparation. Stage at the time of publication: ISO/DIS 22258:2019.

3.3
root diameter
diameter of the tap root, in centimetres

3.4
root top diameter
diameter of the tap root at the stem scar of the tap root, in centimetres

3.5
root mid-diameter
diameter of the tap root at the mid-length of the tap root, in centimetres

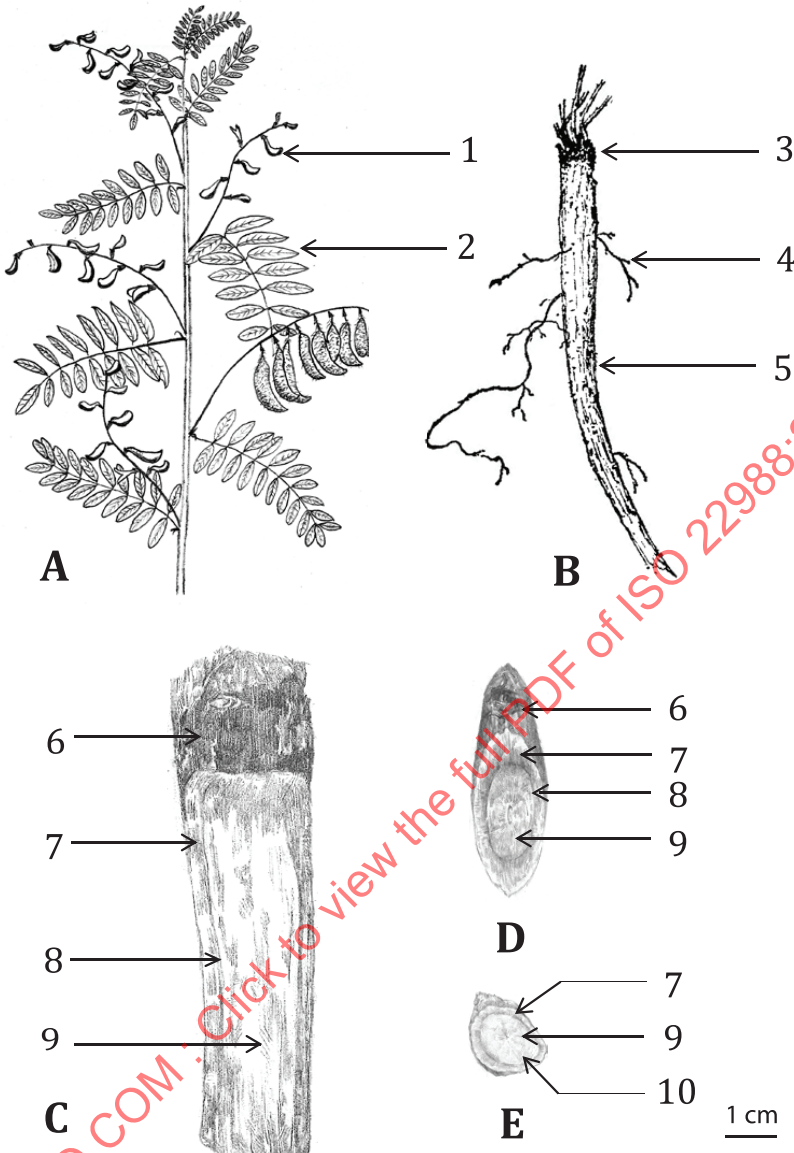
3.6
root bottom diameter
diameter of the tap root at the bottom of the tap root, in centimetres

3.7
marker compound
astragaloside I ($C_{45}H_{72}O_{16}$), astragaloside II ($C_{43}H_{70}O_{15}$), astragaloside IV ($C_{41}H_{68}O_{14}$), calycosin ($C_{16}H_{12}O_5$), calycosin-7-glucoside ($C_{22}H_{22}O_{10}$), formononetin ($C_{16}H_{12}O_4$), and ononin ($C_{22}H_{22}O_9$)

3.8
batch
samples collected from the same particular place at the same time, of no more than 5 000 kg

4 Descriptions

In this document, *Astragalus mongholicus* root is the dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao in the family of Leguminosae shown in Figure 1.



Key

A	plant of *Astragalus mongholicus*	4	fibrous root
B	dried tap root	5	tap root
C	longitudinal section of the tap root	6	epidermis
D	slanting section of the tap root	7	phloem
E	transverse section of the tap root	8	cambium
1	flower	9	xylem
2	leaf	10	radiate striations
3	stem scar		

Figure 1 — Structure of *Astragalus mongholicus* root

5 Requirements

5.1 Morphological features

a) The roots are cylindrical, some branched, upper part relatively thick.

b) The roots are 30 cm to 90 cm long, 1 cm to 3,5 cm in diameter.

c) The outer surface is pale brownish-yellow or pale brown, with irregular, longitudinal wrinkles or furrows.

d) The texture is hard and tenacious, uneasily broken.

e) The fracture is highly fibrous and starchy, bark yellowish-white, wood pale yellow, with radiate striations and fissures, the centre part of old root occasionally rotten wood-shaped, blackish-brown or hollowed.

f) The odour is weak.

g) The taste is slightly sweet and slightly bean-like on chewing.

5.2 Moisture

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

5.3 Total ash

The content of total ash in percentage mass should not be more than 5,0 %.

5.4 Water-soluble extractives

The content of water-soluble extractives in percentage mass should not be less than 17,0 %.

5.5 Thin-layer chromatogram identification

The thin-layer chromatogram (TLC) of *Astragalus mongholicus* root shall present the spots and bands with the same colour and position corresponding to those of reference solutions.

5.6 Total polysaccharides

The content of total polysaccharides in percentage mass shall be determined.

5.7 Marker compounds

The content of marker compounds in percentage mass shall be determined.

For example, saponins (such as astragaloside I, astragaloside II or astragaloside IV) or isoflavonoids (such as calycosin, calycosin-7-glucoside, formononetin or ononin) shall be determined according to relevant national or regional pharmacopoeias.

5.8 Heavy metals

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

5.9 Pesticide residues

The content of pesticide residues shall be determined.

5.10 Grade

The grade of *Astragalus mongholicus* root shall be established only when all four requirements, i.e. root top diameter, root mid-diameter, root bottom diameter and root length, are met. The root diameter and root length of each batch of *Astragalus mongholicus* root shall conform to the requirements in Table 1.

Table 1 — Grading requirements of *Astragalus mongholicus* root

Grade	Root diameter cm			Root length cm
	Root top diameter	Root mid-diameter	Root bottom diameter	
First	> 2	> 2	≥ 0,6	> 70
Second	> 1,5	> 1,5	≥ 0,5	> 50
Third	> 1	> 1	≥ 0,4	> 40
Fourth	> 0,7	> 0,7	≥ 0,3	—
Unqualified	≤ 0,7	≤ 0,7	< 0,3	—

NOTE 1 The requirements are based on roots collected from different production regions of *Astragalus mongholicus* root.

NOTE 2 The grading requirements are established according to the traditional grading system of *Astragalus mongholicus* root that has long been extensively used in the market and trading and that has also been adopted by the national standards of the People's Republic of China.

NOTE 3 Traditionally and practically, the market price of *Astragalus mongholicus* root is positively correlated with the grades. The higher the grade, the more expensive the price in the market.

NOTE 4 The grade merely reflects size and TCM knowledge of the product, which has no clear relationship with its efficacy and safety.

6 Sampling

Sampling shall be carried out in accordance with the method described in the World Health Organization, *Quality control methods for herbal materials*, *General advice on sampling*. Sampling of *Astragalus mongholicus* root shall be conducted according to the following steps:

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

b) from a batch of six units 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 10. 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;

e) the three original samples shall then be combined into a pooled sample which shall be mixed carefully;

f) the average sample is obtained by quartering:

   — mix the pooled sample into an even and square-shaped heap;

   — divide diagonally into four equal parts;

   — take two diagonally opposite parts and mix carefully;

   — 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 measure and analyses specified in Table 2.

Table 2 — Maximum mass of batch and minimum mass of final sample

Maximum mass per batch kg	Minimum mass of final sample g		
	For measure of root mass and root length	For analysis of marker compounds	For other analyses
5 000	500	250	250
NOTE 1 The establishment of the requirement is based on *Astragalus mongholicus* root collected from different producing areas.			
NOTE 2 Other analyses include macroscopic identification, the determinations of moisture, total ash, water-soluble extractives, polysaccharides, heavy metals and pesticide residues, and thin-layer chromatogram identification.			

7 Test methods

7.1 Macroscopic identification

Samples of not less than 250 g are taken from each batch randomly. These samples are examined by naked eye observation, smell and taste.

7.2 Determination of moisture content

See Annex A for additional information.

7.3 Determination of total ash content

The testing method specified in ISO 1575 shall apply.

7.4 Determination of water-soluble extractives content

See Annex B for additional information.

7.5 Thin-layer chromatogram identification

See Annex C for additional information.

7.6 Determination of total polysaccharides content

See Annex D for additional information.

7.7 Determination of marker compound content

The testing method specified in Annex E, the United States Pharmacopoeia[6], the British Pharmacopoeia[1], the Pharmacopoeia of the People's Republic of China[2] and the European Pharmacopoeia[3] can be used.

7.8 Determination of heavy metals content

The testing method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues content

The testing method specified in ISO 22258[3)] shall apply.

3) Under preparation. Stage at the time of publication: ISO/DIS 22258:2019.

7.10 Root diameter

Samples of not less than 500 g are taken from each batch randomly. The diameters of the tap root at the stem scar, mid-length and bottom of the tap root are measured, respectively, one by one. The average top diameter, mid-diameter and bottom diameter of the samples is then calculated, respectively.

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 the samples is then calculated.

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 22988:2020;

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

The storage conditions specified in ISO 22217[4)] shall apply.

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

10 Marking and labelling

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

a) grade of the products in accordance with 5.10;

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

c) gross mass and net mass of the package;

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

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

f) storage and transportation method.

4) Under preparation. Stage at the time of publication: ISO/DIS 22217:2019.

Annex A
(informative)

Determination of moisture content

A.1 Procedure

a) Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Place (2–5) g of the powdered sample in a flat weighing bottle which has been previously dried to constant mass to form a smooth layer not exceeding 10 mm in thickness, and then weigh accurately.

b) Dry in an oven at (100–105) °C for 5 h with the stopper of the bottle removed.

c) Upon opening the oven, close the bottle promptly and allow it to cool in a desiccator for 30 min.

d) Weigh accurately and dry it again under similar conditions for 1 h, cool and weigh.

e) Repeat the operation until the difference between two successive weighings is not more than 5 mg.

A.2 Expression of result

Calculate the mass fraction of water, w_M (%), in the sample being examined according to the mass loss on drying with Formula (A.1):

$$w_M = (m_3 - m_2)/(m_3 - m_b) \times 100 \quad \text{(A.1)}$$

where

m_b is the mass of the flat weighing bottle (g);

m_3 is the mass of the flat weighing bottle with the sample being examined before drying (g);

m_2 is the mass of the flat weighing bottle with the sample being examined after drying (g).

Annex B
(informative)

Determination of water-soluble extractives content

B.1 Procedure

a) Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve.

b) Place 4 g of the accurately weighed powdered sample in a (250–300) ml stoppered conical flask, accurately add 100 ml of water and stopper well.

c) Macerate the sample with shaking for 6 h and allow to stand for 18 h at room temperature.

d) Filter the sample rapidly through a dried filter, accurately transfer 20 ml of the successive filtrate into an evaporating dish, previously dried to constant mass, and evaporate the filtrate to dryness on a water bath.

e) Dry the extract at 105 °C for 3 h and allow to cool for 30 min in a desiccator.

f) Weigh the evaporating dish with the dried extract rapidly and accurately.

B.2 Expression of result

Calculate the mass fraction of water-soluble extractives, w_{wse} (%), of the sample being examined on the dried basis with Formula (B.1):

$$w_{wse} = (m_1 - m_0)5 / m_s \times 100 \quad \text{(B.1)}$$

where

m_s is the mass of the sample being examined (g);

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

m_1 is the mass of the evaporating dish with the extract after drying (g).

Annex C
(informative)

Thin-layer chromatogram identification

C.1 Identification of astragaloside IV in the methanol extract of *Astragalus mongholicus* root

a) Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Add 20 ml of methanol to 3 g of the powdered sample in a 100 ml round-bottom flask, heat under reflux on a water bath for 1 h and filter.

b) Load the filtrate to a neutral aluminium oxide column [(100–120) mesh, 5 g, (10–15) mm in inner diameter] and elute with 100 ml of 40 % methanol, collect the eluate and evaporate it on a water bath to dryness.

c) Dissolve the residue in 30 ml of water and extract with water-saturated n-butanol twice by shaking, each time with 20 ml of n-butanol, combine the n-butanol solutions and wash with water twice by shaking, each time with 20 ml of water.

d) Discard the water solution and evaporate the n-butanol solution to dryness on a water bath.

e) Dissolve the residue in 0,5 ml of methanol as the test solution.

f) Dissolve the chemical reference standard of astragaloside IV in methanol to produce a solution containing 1 mg of astragaloside IV per ml as the reference solution.

g) Carry out the method for thin layer chromatography using silica gel G as the coating substance and the lower layer of a mixture of chloroform, methanol and water (13:7:2) as the mobile phase. Apply 2 µl of each of the above two solutions separately to the silica gel G plate.

h) After developing and removal of the plate, dry the plate in air, spray with a 10 % solution of sulfuric acid in ethanol, and heat at 105 °C until the spots are distinct.

i) Examine under sunlight; the same brown spots in corresponding positions are shown in both chromatograms obtained with the test solution and the reference solution, confirming the presence of astragaloside IV in the root extract being tested.

j) Examine under ultraviolet light at 365 nm; the same orange-yellow fluorescent spots are shown in both chromatograms obtained with the test solution and the reference solution, confirming the presence of astragaloside IV in the root extract being tested.

C.2 Identification of methanol extracts of *Astragalus mongholicus* root comparing with the reference drug

a) Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve, add 30 ml of methanol to 2 g of the powdered sample, heat under reflux for 20 min, filter and evaporate the filtrate to dryness.

b) Dissolve the residue in 15 ml of 0,3 % solution of sodium hydroxide and filter.

c) Adjust the filtrate to pH (5–6) with dilute hydrochloric acid, extract with 15 ml of ethyl acetate by shaking, separate the ethyl acetate solution and filter through a filter paper covered with a quantity of anhydrous sodium sulfate.

d) Evaporate the filtrate to dryness and dissolve the residue in 1 ml of ethyl acetate as the test solution.

e) Prepare a solution of *Astragalus mongholicus* root reference drug in the same manner as above as the herbal reference solution.

e) Carry out the method for thin layer chromatography, using silica gel G as the coating substance and a mixture of chloroform and methanol (or butanol) (10:1) as the mobile phase. Apply 10 µl of each of the above two solutions separately to the silica gel G plate.

f) After developing and removal of the plate, dry the plate in air, expose to ammonia vapour and examine under ultraviolet light at 365 nm.

g) The main fluorescent spots in the chromatogram obtained with the test solution correspond in position and colour to the spots in the chromatogram obtained with the herbal reference drug solution.

Annex D
(informative)

Determination of total polysaccharides content

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 1 g of the powder and transfer to a flask, add 80 ml of water, heat and reflux for 2 h, and cool. Transfer the solution to a 100 ml volumetric flask, rinse the flask three times with 5 ml of water, and transfer the washings to the volumetric flask. Dilute with water to volume, mix and filter. Discard the first portion of the filtrate and transfer 2 ml of the successive filtrate to a 15 ml centrifugal tube. Add 10 ml of ethanol to a centrifuge tube, mix and chill for 1 h. Centrifuge at 4 000 rpm for 20 min, discard the supernatant and wash the precipitate with 8 ml of 80 % ethanol twice. Centrifuge again and discard the supernatant. Dissolve the precipitate with hot water and transfer the solution to a 10 ml volumetric flask, cool, dilute with water to volume and mix well as the test solution.

D.2 Preparation of reference standard solution

Weigh accurately a quantity of anhydrous glucose to a volumetric flask then dissolve in water to prepare a solution containing 90 µg glucose per millilitre as the reference standard solution.

D.3 Construction of calibration curve

Transfer separately 0,2 ml, 0,3 ml, 0,4 ml, 0,5 ml and 0,6 ml of the reference standard solution to 10-ml test tubes with glass stoppers, dilute with water to 2,0 ml, add 1,0 ml of freshly prepared 5 % phenol solution and mix. Add 5,0 ml of sulfuric acid and mix. Heat for 20 min in a boiling water bath and cool the tubes in an ice bath for 5 min. Determine the absorbance of the samples at 488 nm using an ultraviolet-visible spectrophotometer. Construct the calibration curve by plotting the absorbance (y-axis) against the concentration of the glucose (x-axis).

D.4 Determination of polysaccharides

Transfer 2,0 ml of the sample solution to a 10-ml test tube with glass stopper and determine the absorbance according to method in D.3 (begin from 'add 1,0 mL of freshly prepared 5 % phenol solution'). Calculate the content of glucose in test solutions by the calibration curve. The mass fraction of polysaccharides of the sample being examined on the dried basis, w_{pol} (%), is calculated with Formula (D.1):

$$w_{pol} = (a-b)20\times250/c\times m_s 10^3\times100 \quad \text{(D.1)}$$

where

a is the absorbance of the test solution;

b is the intercept of the calibration curve;

c is the slope of the calibration curve;

m_s is the mass of the sample being examined.

Annex E
(informative)

Determination of marker compound content

E.1 Preparation of test solution

Weigh 250 g of sample to grind and pass it through an 80 mesh or finer sieve. Weigh accurately 1,0 g of the powdered sample to a conical flask, accurately add 50 ml of methanol and weigh. Reflux in a water bath below 75 °C for 2 h. Cool and weigh again. Replenish the loss of solvent with methanol and mix well. Filter and use the successive filtrate. Further filter through a 0,2-µm membrane filter and use the filtrate as the test solution.

E.2 Preparation of reference solution

Weigh accurately reference substance(s) below as needed, dissolve in the proper amount of methanol to produce a mixture as reference solution with concentrations as shown in Table E.1.

Table E.1 — Reference solution

Compound	Concentration mg/ml
Astragaloside I ($C_{45}H_{72}O_{16}$)	0,30
Astragaloside II ($C_{43}H_{70}O_{15}$)	0,40
Astragaloside III ($C_{41}H_{68}O_{14}$)	0,20
Astragaloside IV ($C_{41}H_{68}O_{14}$)	5,00
Calycosin ($C_{16}H_{12}O_5$)	0,20
Calycosin-7-Glucoside ($C_{22}H_{22}O_{10}$)	0,50
Formononetin ($C_{16}H_{12}O_4$)	0,30
Ononin ($C_{22}H_{22}O_9$)	0,20

E.3 Chromatographic conditions

E.3.1 Stationary phase: octadecylsilane bonded silica gel or equivalent as analysing column.

E.3.2 Size: l = 100 mm, Ø = 2,1 mm.

E.3.3 Mobile phase.

E.3.3.1 Mobile phase A: 0,1 % formic acid.

E.3.3.2 Mobile phase B: acetonitrile.

E.3.3.3 Program of gradient elution as shown in Table E.2.

Table E.2 — Typical chromatographic conditions

Time min	Mobile phase A volumic fraction (%)	Mobile phase B volumic fraction (%)
0	80	20
7	50	50
8	50	50
10	10	90

E.3.4 Flow rate: 0,35 ml/min.

E.3.5 Injection volume: 2µl.

E.3.6 Temperature of column oven: 30 °C.

E.4 Mass spectrometric conditions

E.4.1 Typical mass spectrometric conditions are shown in Table E.3.

Table E.3 — Typical mass spectrometric conditions

Ionization mode	ESI positive
Mode	Multiple reaction monitoring (MRM)
Drying gas	N_2
Flow rate of drying gas	11,0 l/min
Drying gas temperature	300 °C
Nebulizer pressure	15 psig
Capillary voltage	4 kV

E.4.2 Ions monitored are shown in Table E.4.

Table E.4 — Ions monitored

Compound	Parent ion (m/z)	Daughter ion (m/z)
Astragaloside I ($C_{45}H_{72}O_{16}$)	869,5	143,1
Astragaloside II ($C_{43}H_{70}O_{15}$)	827,5	143,1
Astragaloside III ($C_{41}H_{68}O_{14}$)	785,5	143,1
Astragaloside IV ($C_{41}H_{68}O_{14}$)	785,5	143,1
Calycosin ($C_{16}H_{12}O_5$)	285	143,1
Calycosin-7-Glucoside ($C_{22}H_{22}O_{10}$)	447,1	285,1
Formononetin ($C_{16}H_{12}O_4$)	269,1	197,7
Ononin ($C_{22}H_{22}O_9$)	430,1	285,1