



**International
Standard**

ISO 13615

**Traditional Chinese medicine —
Atractylodes macrocephala rhizome**

*Médecine traditionnelle chinoise — Rhizome d'Atractylodes
macrocephala*

**First edition
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Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Descriptions	2
5 Quality and safety requirements and recommendations	3
5.1 General characteristics	3
5.2 Morphological features	3
5.2.1 <i>Atractylodes macrocephala</i> rhizome	3
5.2.2 Decoction pieces	3
5.3 Identification	3
5.3.1 Thin-layer chromatography (TLC) identification	3
5.3.2 High performance liquid chromatography (HPLC) identification	3
5.4 Moisture	3
5.5 Total ash	4
5.6 Sulfur dioxide residues	4
5.7 Ethanol-soluble extractives	4
5.8 Essential oil	4
5.9 Heavy metals	4
5.10 Pesticide residues	4
6 Sampling	4
7 Test methods	4
7.1 Macroscopic identification	4
7.2 Thin-layer chromatography (TLC) identification	4
7.3 High performance liquid chromatography (HPLC) identification	4
7.4 Determination of moisture content	4
7.5 Determination of total ash content	4
7.6 Determination of sulfur dioxide residues content	4
7.7 Determination of ethanol-soluble extractives content	5
7.8 Determination of essential oil content	5
7.9 Determination of heavy metals content	5
7.10 Determination of pesticide residues content	5
8 Test report	5
9 Packaging, storage and transportation	5
10 Marking and labelling	5
Annex A (informative) Thin-layer chromatography (TLC) identification	6
Annex B (informative) High performance liquid chromatography (HPLC) identification	8
Annex C (informative) Reference values of national and regional limits	11
Bibliography	12

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 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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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

Atractylodes macrocephala rhizome, the dried rhizome of *Atractylodes macrocephala* Koidz., is a widely used herbal medicine in China and many other countries. In traditional Chinese medicine, this herb can tonify the Spleen-Qi, and is used to treat distension in the abdomen, loose stools, and diarrhoea. Modern pharmacological studies also demonstrate its great potential in immuno-enhancement and indigestion treatment. The market, yield, and trade volume of *Atractylodes macrocephala* rhizome are large and develop rapidly in recent years.

Atractylodes macrocephala rhizome has been recorded in several pharmacopoeias, including Chinese Pharmacopoeia^[1], European Pharmacopoeia^[2], Korean Pharmacopoeia^[3], Japanese Pharmacopoeia^[4] and Hong Kong Chinese Materia Medical Standards^[5]. The requirements and specifications in these standards have differences. Therefore, it is important to develop an International Standard for harmonizing the existing standards, as well as ensuring the safety and effectiveness of *Atractylodes macrocephala* rhizome, which can benefit farmers, consumers, enterprises and governments involved in the cultivation, regulation and trade of *Atractylodes macrocephala* rhizome.

As national implementation may differ, national standards bodies are invited to modify the values given in [5.4](#) and [5.5](#) in their national standards. Examples of national and regional values are given in [Annex C](#).

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Traditional Chinese medicine — *Atractylodes macrocephala* rhizome

1 Scope

This document specifies requirements and test methods for *Atractylodes macrocephala* rhizome that is derived from *Atractylodes macrocephala* Koidz.

It is applicable to *Atractylodes macrocephala* rhizome that is sold and used as a natural medicine in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces derived from the plant.

2 Normative references

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

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

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

ISO 22217, *Traditional Chinese medicine — Storage requirements for raw materials and decoction pieces*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23723, *Traditional Chinese medicine — General requirements for herbal raw material and materia medica*

3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

***Atractylodes macrocephala* rhizome**

dried rhizome (3.2) of *Atractylodes macrocephala* Koidz in the family of Atractylodes.

3.2

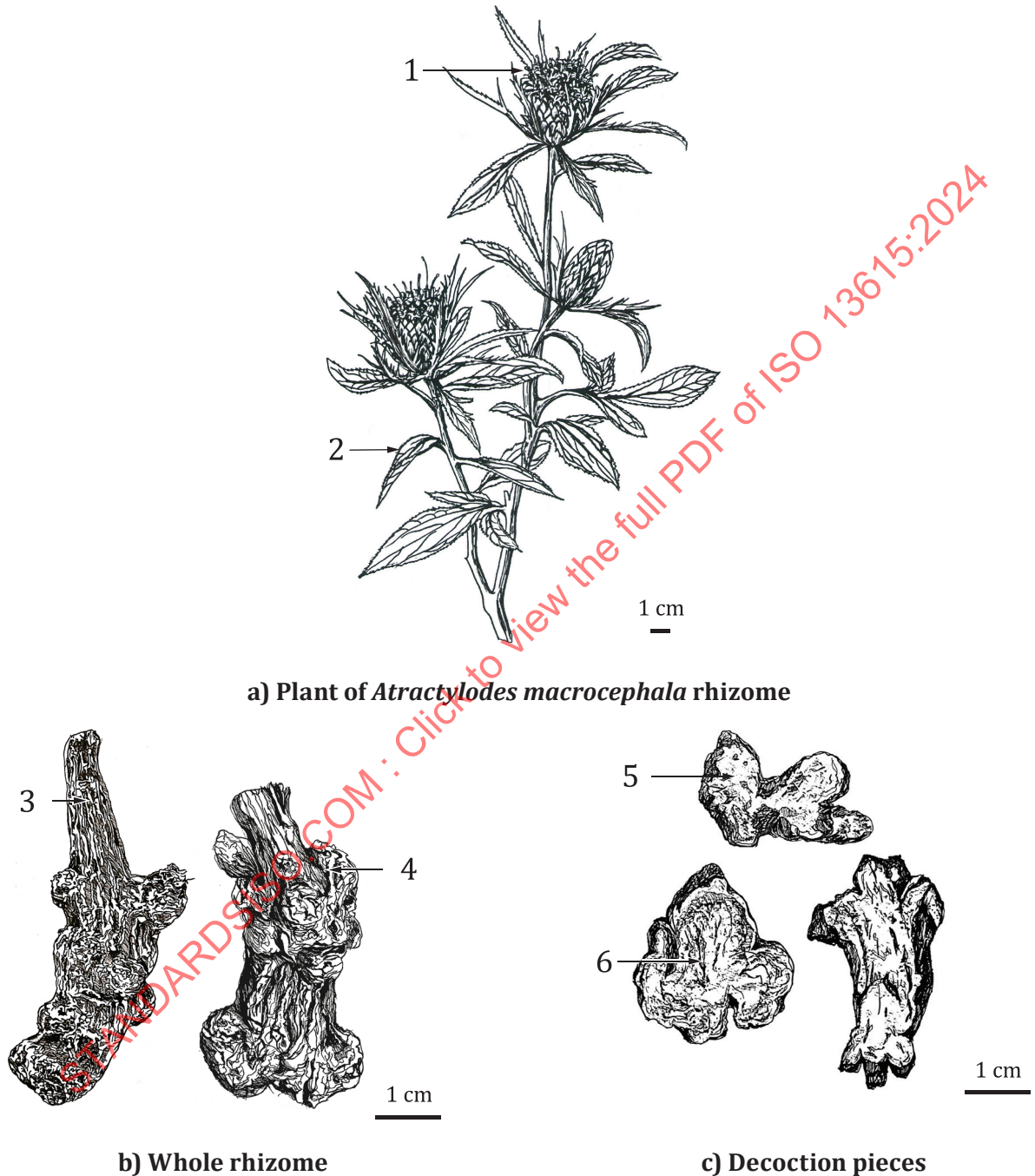
rhizome

creeping rootstalk

main stem of a plant that sends out roots and shoots from its nodes

4 Descriptions

[Figure 1](#) illustrates the structure of *Atractylodes macrocephala* rhizome. The crude drug is collected in winter, when the leaves at the lower part of the plant turn yellow and those at the upper part become fragile; with soil removed, it is baked over low heat or dried under the sun; the fibrous roots are removed to obtain *Atractylodes macrocephala* rhizome.



Key

- 1 flower
- 2 foliage
- 3 wrinkles and grooves

- 4 warty protrudings
- 5 dotted oil cavities
- 6 chrysanthemum-shaped cracks

Figure 1 — Structure of *Atractylodes macrocephala* rhizome

5 Quality and safety requirements and recommendations

5.1 General characteristics

The following requirements shall be met before sampling.

- a) *Atractylodes macrocephala* rhizome shall be clean and free from foreign matter.
- b) The presence of living insects, moulds and external contaminants which are visible to the naked eye shall not be permitted.

5.2 Morphological features

5.2.1 *Atractylodes macrocephala* rhizome

Atractylodes macrocephala rhizomes are irregularly plump masses, frog, drumstick or ruyi-shaped, 3 cm to 13 cm long and 1,5 cm to 7 cm in diameter. The external colour is greyish yellow or greyish brown, with warty protrudings, interrupted longitudinal wrinkles and grooves, and scars of fibrous rootlets; remains of stems and bud scars are attached to the apex. The texture is hard, not easily broken; the fracture is uneven, yellowish-white to pale brown, scattered with obvious brownish-yellow dotted oil cavities. The bake-dried material is horny and relatively dark coloured or cracked in section view. The odour is aromatic. The taste is sweet and slightly pungent. It gets viscous when chewed.

5.2.2 Decoction pieces

The decoction pieces are irregular thick slices. The external is greyish yellow or greyish brown. The cut surface is yellowish-white to pale brown, scattered with obvious brownish-yellow dotted oil cavities, xylem with radial striations; the cut surface is horny and with deeper colour and clefts when dried by oven. The odour is faintly aromatic. The taste is sweet and slightly pungent. It gets slightly viscous when chewed.

5.3 Identification

5.3.1 Thin-layer chromatography (TLC) identification

The identification of *Atractylodes macrocephala* rhizome by thin-layer chromatogram (TLC) should present spots or bands with the same colour and positions corresponding to those of the reference solution. Furthermore, other faint zones may be present in the chromatogram obtained with the test solution.

5.3.2 High performance liquid chromatography (HPLC) identification

When TLC bands are not clearly identified, HPLC identification can be applied. The chromatogram of the test solution should exhibit 3 characteristic peaks (atractylenolide II, atractylenolide III and atractylon), corresponding in the retention times to those in chromatogram of the reference solution.

5.4 Moisture

The moisture content should not be more than 15,0 %.

5.5 Total ash

The total ash content should not be more than 7,0 %.

5.6 Sulfur dioxide residues

The content of sulfur dioxide residues should be determined.

5.7 Ethanol-soluble extractives

The ethanol-soluble extractives content should be determined.

5.8 Essential oil

The essential oil content should be determined.

5.9 Heavy metals

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

5.10 Pesticide residues

The contents of pesticide residues such as hexachlorocyclohexane (BHC), dichloro-diphenyl-trichloroethane (DDT) and quintozone should be determined.

6 Sampling

Sampling of *Atractylodes macrocephala* rhizome shall be in accordance with ISO 23723.

7 Test methods

7.1 Macroscopic identification

The samples are examined by the naked eye, smelled and tasted.

7.2 Thin-layer chromatography (TLC) identification

See [Annex A](#) for additional information on thin-layer chromatography (TLC) identification.

7.3 High performance liquid chromatography (HPLC) identification

See [Annex B](#) for additional information on high performance liquid chromatography (HPLC) identification.

7.4 Determination of moisture content

The test method specified in ISO 23723 shall apply.

7.5 Determination of total ash content

The test method specified in ISO 23723 shall apply.

7.6 Determination of sulfur dioxide residues content

The test method specified in ISO 22590 shall apply.

7.7 Determination of ethanol-soluble extractives content

The test methods specified in ISO 23723 shall apply (hot extraction).

7.8 Determination of essential oil content

The test method specified in ISO 23723 shall apply.

7.9 Determination of heavy metals content

The test method specified in ISO 18664 shall apply.

7.10 Determination of pesticide residues content

The test method specified in ISO 22258 shall 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 operating details not specified in this document, or regarded as optional, together with details of any incidents which can 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 flavour or odour to the product and shall not contain substances which can damage the product or constitute a health risk.

The method specified in ISO 22217 shall apply for the storage of *Atractylodes macrocephala* rhizome.

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

10 Marking and labelling

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

- a) product name and plant scientific name;
- b) all quality features indicated in [Clause 5](#);
- c) country and province/state of origin of the products;
- d) date of production, batch number and expiry date of the products.

Annex A (informative)

Thin-layer chromatography (TLC) identification

A.1 Preparation of test solution

Weigh 1,0 g of the powder, add 2 ml of methanol, ultrasonicate extract for 15 min, filter, take the filtrate as the test solution.

A.2 Preparation of reference solution

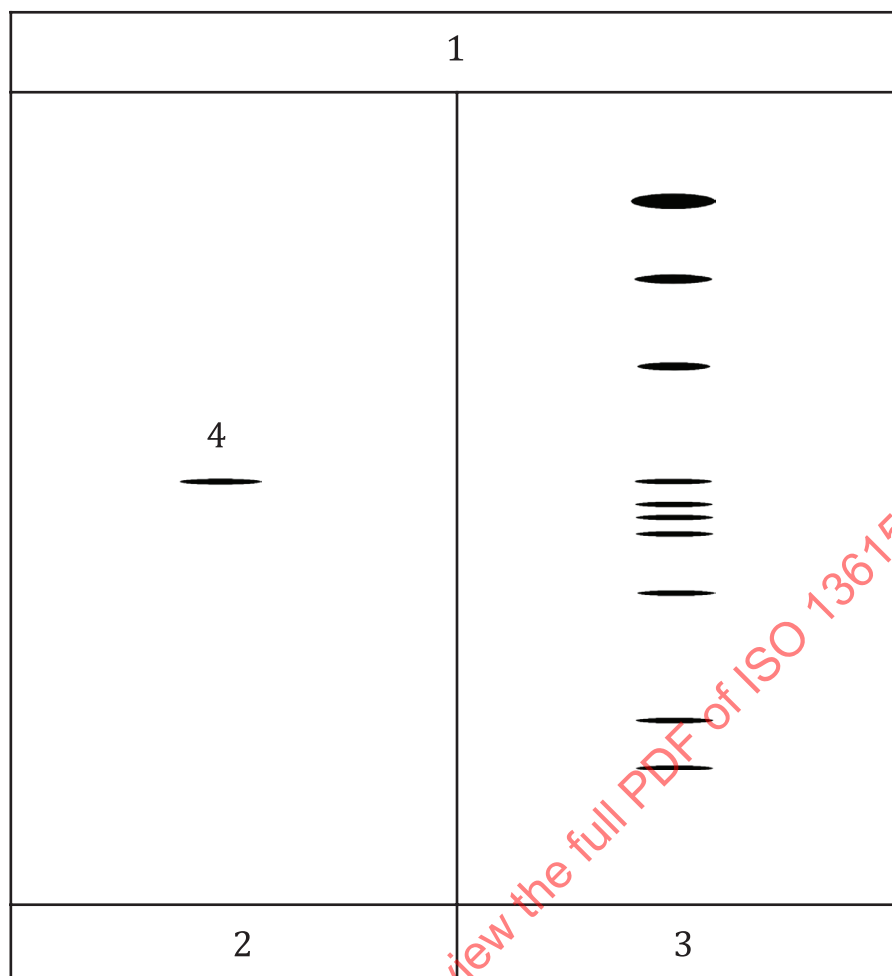
Weigh the appropriate amount of atractylenolide II, add methanol to prepare a solution containing 0,1 mg of atractylenolide II per millilitre as the reference solution.

A.3 Developing solvent system

Prepare a mixture of cyclohexane and 2-propanol (9:1, volume fraction) as the mobile phase.

A.4 Procedure

Use silica gel G as the adsorbent for the thin-layer plate. Apply separately to the plate 3 µl of the test solution and the reference solution. After developing and removal of the plate, dry in air, and spray with a 10 % sulfuric acid in ethanol, heat for 1 min and examine under ultraviolet light at 365 nm. The spots in the chromatogram obtained with the test solution correspond in position and colour to the spots in the chromatogram obtained with the reference solution. Typical TLC chromatograms are shown in [Figure A.1](#).



Key

- 1 top of the plate
- 2 reference solution
- 3 test solution
- 4 atractylenolide II

Figure A.1 — Typical TLC chromatograms of *Atractylodes macrocephala* rhizome

Annex B (informative)

High performance liquid chromatography (HPLC) identification

B.1 Preparation of test solution

Weigh 2 g of the powder (through a 24 mesh or finer sieve), add 10 ml of methanol, let the solution stand for 2 h, then ultrasonicate extract for 30 min, centrifuge at 4 000 r/min for 10 min. Take the supernatant and transfer it to a 50 ml volumetric flask, repeat the extraction twice, and combine the extracts, add methanol to the mark, shake well. Before injection, pass through a filter having a 0,45 µm pore size, discard the first portion of the filtrate, and take the subsequent filtrate as the test solution.

B.2 Preparation of reference solution

Weigh a quantity of atractylenolide III to a measuring flask, dissolve in methanol to prepare a solution containing 0,1 mg of atractylenolide III per millilitre as the reference solution.

B.3 Apparatus and chromatographic conditions

B.3.1 Column:

Stationary phase: octadecylsilyl silica gel for chromatography (5 µm).

Size: $l = 250$ mm, $\varnothing = 4,6$ mm.

B.3.2 Detector: UV 220 nm.

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

B.3.4 Injection volume: 5 µl.

B.3.5 Mobile phase

B.3.5.1 Mobile phase A: acetonitrile.

B.3.5.2 Mobile phase B: water.

B.3.5.3 Gradient program as shown in [Table B.1](#).

Table B.1 — Gradient program

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	20	80
5	20	80
10	60	40
25	90	10
30	90	10
32	100	0
40	100	0

NOTE There was no significant effect of column temperature in the range of 20 °C to 30 °C, injection volume in the range of 5 µl to 10 µl, flow rate in the range of 0,8 ml/min to 1,2 ml/min and detection wavelength in the range of 215 nm to 225 nm by durability examination.

B.4 Detection of atractylenolide II, atractylenolide III and atractylon

Inject 5 µl of the reference solution and the test solution for chromatographic analysis, respectively. The approximate relative retention times, relative to atractylenolide III, are provided in [Table B.2](#) to identify the peak of atractylenolide II and atractylon.

Table B.2 — Relative retention time and correction factor of atractylenolide II, atractylenolide III and atractylon

Tested ingredients	Relative retention time	Correction factor (<i>f</i>)
Atractylenolide III	1,00	1,00
Atractylenolide II	1,21	0,67
Atractylon	1,83	1,54

Separately calculate the percentage of atractylenolide II, atractylenolide III and atractylon in the sample. The content is calculated with [Formula \(B.1\)](#):

$$W = f \times \frac{A_x \times C_s \times 50}{A_s \times 10^3} \times \frac{1}{m_s \times (1 - w_M)} \times 100 \quad (\text{B.1})$$

where

f is the correction factor;

A_x is the peak area of the compound in the test solution;

A_s is the peak area of atractylenolide III in the reference solution;

C_s is the concentration of atractylenolide III in the reference solution (mg/ml);

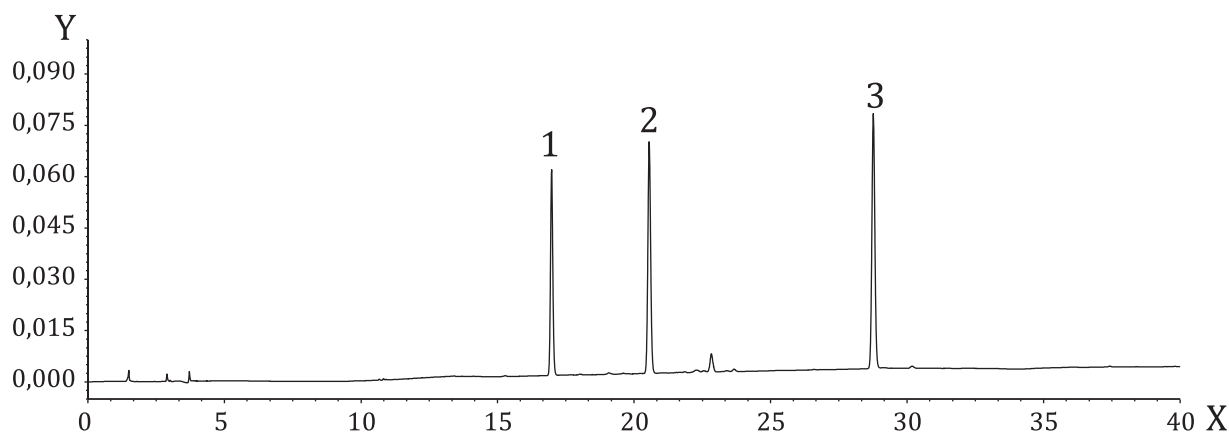
m_s is the mass of the sample (g);

w_M is the moisture content of the sample (%);

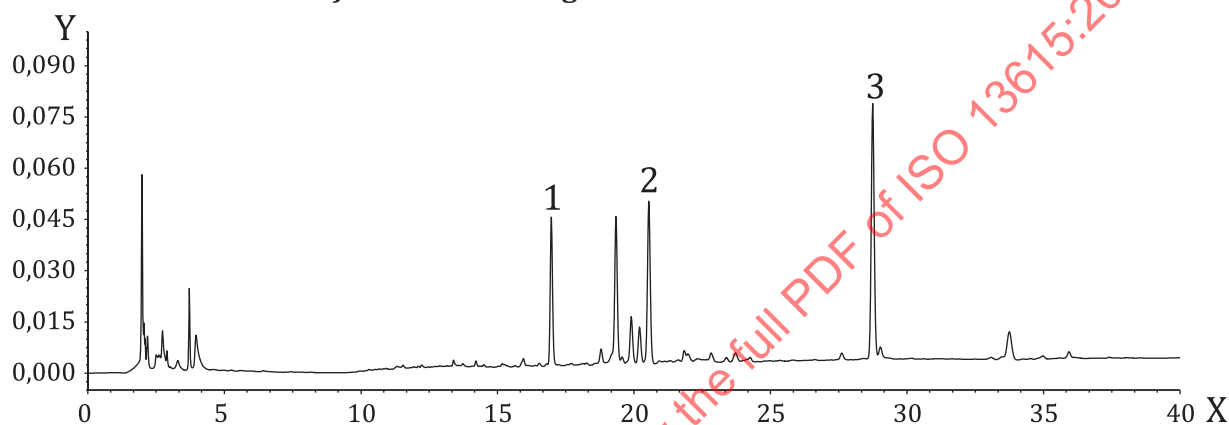
W is the content of atractylenolide II, atractylenolide III or atractylon (%) in the sample.

B.5 Typical HPLC chromatograms

Typical HPLC chromatograms of *Atractylodes macrocephala* rhizome are shown in [Figure B.1](#).



a) HPLC chromatogram of reference solution



b) HPLC chromatogram of test solution

Key

- X min
- Y AU
- 1 atractylenolide III
- 2 atractylenolide II
- 3 atractylon

Figure B.1 — Typical HPLC chromatograms of *Atractylodes macrocephala* rhizome