INTERNATIONAL STANDARD

**ISO 23193**

First edition
2020-08

# Traditional Chinese medicine — *Lycium barbarum* and *Lycium chinense* fruit

*Médecine traditionnelle chinoise — Baie de goji (baie de* Lycium barbarum *et de* Lycium chinense*)*



Reference number
ISO 23193:2020(E)



ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

# 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 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

*Lycium barbarum* and *Lycium chinense* fruit, commonly called Lycium fruit or *Lycii Fructus*, is the dried fruit of *Lycium barbarum* Linné or *Lycium chinense* Mill. (Fam. Solanaceae). Lycium fruit was firstly recorded in the book '*Divine Farmer's Classic of Materia Medica*', and it has a long history in China, Korea, Japan and other Southeast Asian nations, where it is used to nourish the liver and kidneys and replenish essence to improve vision. Clinically, owing to its medicinal properties, it plays an important role in the treatment of diseases such as immune suppression, cancer and diabetic retinopathy.

Additionally, *Lycium barbarum* and *Lycium chinense* fruit, with its sweet taste and warming property, is widely used in functional food and cosmetics. Lycium fruit and its finished products also have a very high reputation worldwide for their effectiveness, and account for a large market share in the international trade of Chinese herbal medicines.

*Lycium barbarum* and *Lycium chinense* fruit is widely cultivated in the northwest of China, Korea and Canada, among other places. However, the quality of Lycium fruit provided from different areas or by different cultivators is quite different. In addition, though *Lycium barbarum* and *Lycium chinense* fruit has been recorded in several pharmacopeia and standards, specifications and quality requirements in these standards vary. Thus, there is a clear and urgent need to develop an international standard for harmonizing the existing standards, as well as ensuring the safety and effectiveness of *Lycium barbarum* and *Lycium chinense* fruit.

As national implementation may differ, national standards bodies are invited to modify the values given in 5.3, 5.4, 5.5, 5.6, 5.8 and Clause 9 in their national standards. Examples of national and regional values are given in Annex E.

# Traditional Chinese medicine — *Lycium barbarum* and *Lycium chinense* fruit

## 1 Scope

This document specifies the minimum requirements and test methods for *Lycium barbarum* and *Lycium chinense* fruit, which is derived from the plant of *Lycium barbarum* L. or *Lycium chinense* Mill.

It is applicable to *Lycium barbarum* and *Lycium chinense* fruit that is sold and used as herbal raw materials in the 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 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*

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*

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*

World Health Organization *Quality control methods for herbal materials,* 2011

## 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**
***Lycium barbarum* fruit**
dried ripe fruit of *Lycium barbarum* L. (Fam. Solanaceae)

**3.2**
***Lycium chinense* fruit**
dried ripe fruit of *Lycium chinense* Mill. (Fam. Solanaceae)

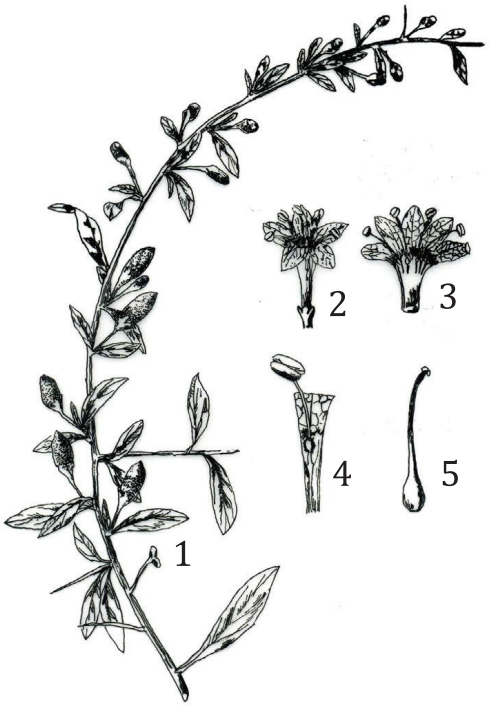
**3.3**
**batch**
samples collected from the same particular place at the same time, of no more than 1 000 kg

**3.4**
**final sample**
samples for the test required in this document

Note 1 to entry: Final samples may be packed in different materials meeting conditions for specific tests (e.g. moisture or total ash).

## 4 Descriptions

The structure of *Lycium barbarum* L., *Lycium chinense* Mill. and the dried ripe fruit are shown in Figure 1. Different features such as leaves, flowers and fruits in *Lycium barbarum* L. and *Lycium chinense* Mill., and methods for differentiating these two species, are given in Annex F.




a) Plant of *Lycium barbarum* L.


b) Plant of *Lycium Chinese* Mill.


c) Dried ripe fruits (upper: *Lycium barbarum* fruit; lower: *Lycium chinense* fruit)

**Key**

1 fruit spur
2 flower
3 corolla expended to show stamens
4 stamen
5 pistil
6 pistil stalk scar
7 fruit stalk scar

**Figure 1 — Structure of *Lycium barbarum* and *Lycium chinense* fruit**

# 5 Requirements

## 5.1 Morphological features

### 5.1.1 Appearance

The fruit is nearly fusiform or elliptical. Pericarp is soft and externally roughly wrinkled. Sarcocarp is pulpy, soft and tender.

### 5.1.2 Colour

The external surface is red or dark red with a pistil stalk scar (6) at the apex and a white fruit stalk scar (7) at the base, as in Figure 1 c).

### 5.1.3 Dimensions

The fruit is 6 mm to 20 mm in length measured from the base to the end of the fruit and 3 mm to 10 mm in diameter measured at the base of the fruit.

### 5.1.4 Fracture

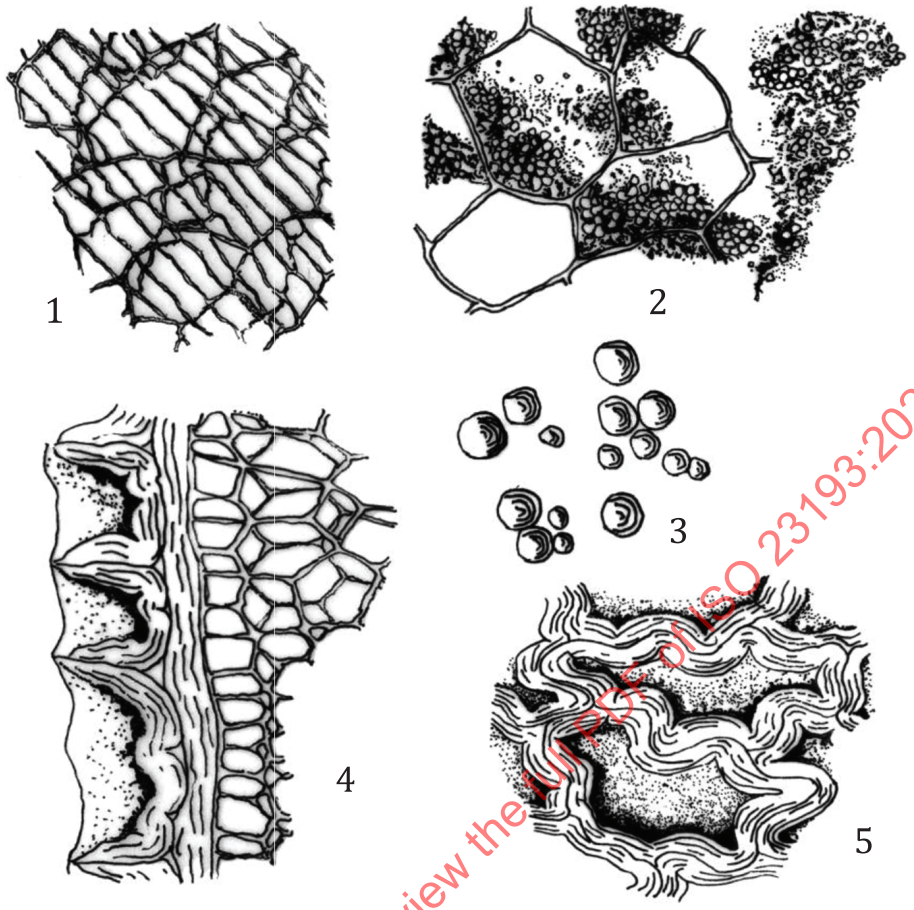
20 to 50 seeds are present inside one fruit, kidney-shaped and nearly flat, ca. 2 mm; the external surface of the seeds is pale yellow or yellowish brown in the fruit of *Lycium barbarum* L., while the seeds in one fruit of *Lycium chinense* Mill. are numerous, kidney-shaped and nearly flat, 2,5 mm to 3 mm; the external surface colour of the seeds is yellow.

### 5.1.5 Odour

The odour is slight, and the taste is at first sweet and then slightly bitter for *Lycium chinense* fruit, and sweet and not bitter for *Lycium barbarum* fruit.

## 5.2 Microscopic characteristics

See Figure 2. The powder is yellowish-orange or reddish-brown. Epidermal cells of outer pericarp (1) are polygonal or elongated-polygonal, about 60 µm in diameter, with straight or slightly wavy walls, covered with a thick cuticle, with distinct, more-or-less parallel striations. Parenchymatous cells of mesocarp (2) are thin-walled, subpolygonal, containing reddish-orange or brownish-red spherical aleurone granules (3). Fragments of starchy endosperm cells (4) contain oil droplets. Stone cells of testa (5) are irregular polygonal, with distinct striations, thickened or slightly wavy walls.




**Key**

1 epidermal cells of outer pericarp
2 parenchymatous cells of mesocarp
3 aleurone granules
4 starchy endosperm cells
5 stone cells of testa

**Figure 2 — Structure of powdered Lycium fruit**



### 5.3 Moisture

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

### 5.4 Total ash

The mass fraction of total ash should not be more than 6,0 %.

### 5.5 Acid-insoluble ash

The mass fraction of total ash should not be more than 1,0 %.

### 5.6 Water-soluble extractives

The mass fraction of water-soluble extractives should not be less than 45,0 %.

### 5.7 Thin-layer chromatogram (TLC) identification

The identification of *Lycium barbarum* and *Lycium chinense* fruit with TLC shall present spots or brands obtained from the test and reference drug solution in the same position with the same colour.

### 5.8 Content of polysaccharide

The content of polysaccharide should be determined.

### 5.9 Content of marker compound

The content of marker compound such as Betaine shall be determined.

### 5.10 Heavy metals

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

### 5.11 Pesticide residues

The content of pesticide residues such as Benzex, DDT and quintozene shall be determined.

### 5.12 Sulfur dioxide residues

The content of sulfur dioxide residues shall be determined.

## 6 Sampling

Sampling of *Lycium barbarum* and *Lycium chinense* fruit shall be in accordance with the World Health Organization's *Quality control methods for herbal materials.*

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

b) From a batch of six 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. 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 this 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 shall be tested for the measurement and analyses specified in Table 1.



**Table 1 — Maximum weight of batch and minimum weight of the final sample**

| Maximum weight of fruit per batch<br>kg | Minimum weight of the final sample<br>g | | |
|---|---|---|---|
| | For macroscopic identification | For determination of marker compound | For other analyses |
| 1 000 | 500 | 250 | 250 |
| NOTE 1 The requirements are based on samples collected from different production regions of *Lycium barbarum* and *Lycium chinense* fruit. | | | |
| NOTE 2 Other analyses include the determination of moisture content, total ash, acid-insoluble content, water-soluble extractives, heavy metals and pesticide residues. | | | |

# 7 Test methods

## 7.1 Macroscopic identification

Samples of not less than 500 g shall be taken from each batch randomly. These samples shall be examined by naked eye observation, smell and taste.

## 7.2 Determination of moisture content

The testing method specified in ISO 20409 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 water-solution extractives content

See Annex A for additional information.

## 7.6 TLC identification

See Annex B for additional information.

## 7.7 Determination of polysaccharide content

See Annex C for additional information.

## 7.8 Determination of marker compound content

See Annex D for additional information.

## 7.9 Determination of heavy metals contents

The testing method specified in ISO 18664 applies.

## 7.10 Determination of pesticide residues contents

The testing method specified in ISO 22258 applies.

### 7.11 Determination of sulfur dioxide residues contents

The testing method specified in ISO 22590 applies.

## 8 Test report

For each test method, the test report shall specify the following:

a) all the information necessary for the complete identification of the sample;

b) the sampling method used;

c) the test method used;

d) a reference to this document, i.e. ISO 23193:2020,

e) the test result(s) obtained;

f) 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);

g) any unusual features (anomalies) observed during the test;

h) the date of the test.

## 9 Packaging, storage and transportation

Packaging should 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 product shall be sealed and stored in a dry, shady and cool place. The storage temperature should be no higher than 25 °C.

The *Lycium barbarum* and *Lycium chinense* fruit shall be protected from light, moisture, pollution and entry of foreign substances during long-distance delivery.

## 10 Marking and labelling

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

a) product name and Latin scientific name;

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

c) gross weight and net weight of the products;

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 methods;

g) any items required by the regulatory bodies of the destination country.

# Annex A
(informative)

# 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 weight. Weigh approximately 4 g of the dried powder into a 250 ml stopper conical flask. Accurately add 100 ml 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 within the first 6 h, then filter rapidly with a dry 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 water-soluble extractives, $m_{wse}$, on the dried basis (%) with Formula (A.1).

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

where

$m_s$ is the mass of the sample (g);

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

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

# Annex B
(informative)

# TLC identification

## B.1 Preparation of test solution

Respectively weigh 250 g of *Lycium barbarum* and *Lycium chinense* fruit to grind and pass it through an 80-mesh or finer sieve. Weigh approximately 0,5 g of the powder, add 35 ml of water, reflux for 15 min and filter. Extract the filter with 15 ml of ethyl acetate three times and evaporate the extracted solution to dryness. Subsequently dissolve the residue with 1 ml of methanol as the sample solution.

## B.2 Preparation of reference solution

Respectively weigh 0,5 g of *Lycium barbarum* and *Lycium chinense* fruit reference drug and treat it in the same manner as in B.1 as the reference drug solution.

## B.3 Developing solvent system

Prepare a mixture of ethyl acetate and dichloromethane with a volume ratio of 2:15 as the mobile phase.

## B.4 Procedure

Apply 5,0 µl each of the reference drug solution and the test solution on the same TLC plate (silica gel G) previously dried at 110°C for 15 min in the oven. Develop the plate in the mobile phase of ethyl acetate and dichloromethane (2:15 volume ratio), then remove the plate and dry in air. Examine the plate under ultraviolet light at 365 nm. Identify the spots of the test solution by comparing the positions and colours with those of the reference drug solution.

A typical reference TLC chromatogram is shown in Figure B.1.

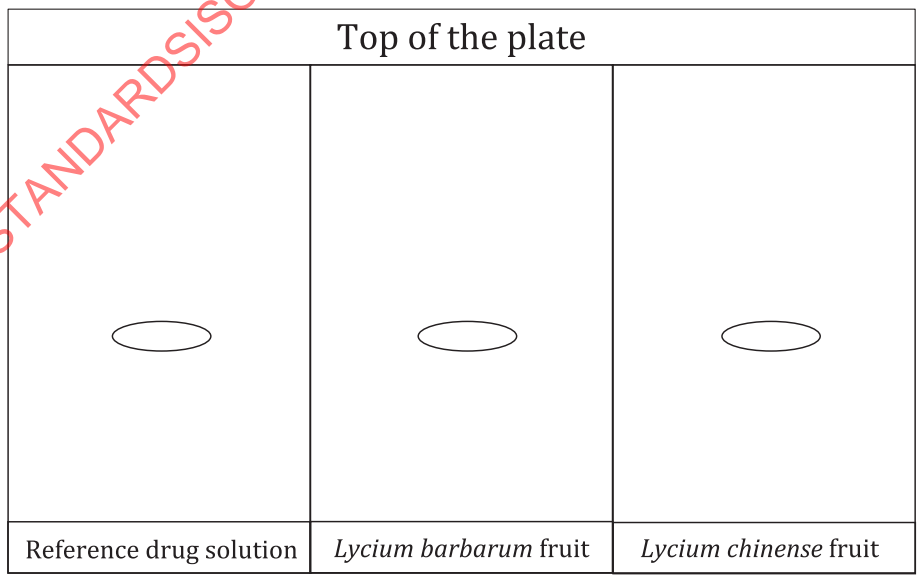



**Figure B.1 — Schematic diagram of typical TLC chromatogram of *Lycium barbarum* and *Lycium chinense* fruit**

# Annex C
(informative)

# Determination of polysaccharide content

## C.1 Principle of the test method

The phenol-sulfuric acid method is employed to determine the content of polysaccharides. In this method, the concentrated sulfuric acid breaks down the polysaccharides to monosaccharides. Pentoses (5-carbon monosaccharides) are then dehydrated to furfural, and hexoses (6-carbon monosaccharides) to hydroxymethyl furfural. These compounds then react with phenol to produce orange-yellow complexes that can be measured spectrophotometrically. Glucose is generally used as the reference standard in this method. Therefore, in this method, the content of the monosaccharide (glucose) can reflect the content of polysaccharides.

## C.2 Preparation of reference standard solution

Accurately weigh 25 mg of anhydrous glucose to a 250 ml volumetric flask, add water to dissolve, dilute to volume, and mix well (containing 0,1 mg anhydrous glucose per ml).

## C.3 Preparation of test solution

Accurately weigh 0,5 g of *Lycium barbarum* and *Lycium chinense* fruit to flask, add 200 ml of water, heat and reflux for 2 h then cool. Transfer the solution to a 250 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 filtrate to a 15 ml centrifugal tube. Add 10 ml of alcohol to the centrifugal 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 % alcohol twice. Centrifuge again and discard the supernatant. Dissolve the precipitate with hot water and transfer the solution to a 25 ml volumetric flask, cool, dilute with water to volume and mix.

## C.4 Construction of calibration curve

Transfer separately 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml and 1,0 ml of the standard solution to 10 ml test tubes with glass stoppers, dilute with water to 1,0 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 tube 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).

## C.5 Content of polysaccharides

Transfer 1,0 ml of the sample solution to a 10 ml test tube with a glass stopper and determine the absorbance according to the preparation of the test method in C.4 (beginning from “add 1,0 ml of freshly

prepared 5 % phenol solution"). Calculate the content of glucose in sample solutions by the calibration curve. The content of polysaccharides, $W_{pol}$ (%), is calculated with Formula (C.1):

$$W_{pol} = \frac{\frac{(a-b)}{c} \times 250 \times 25}{m_s \times 2 \times 10^6 \times (1 - W_m)} \quad \text{(C.1)}$$

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 (g);

$W_m$ is the moisture content of the sample (%).

# Annex D
(informative)

# Determination of betaine content

## D.1 Preparation of reference standard solution

Dissolve a quantity of betaine CRS with methanol in a brown cliometric flask to produce a solution containing 0,1 mg of each per ml as the reference solution.

## D.2 Preparation of test solution

2,0 g of the fine-grained *Lycium barbarum* and *Lycium chinense* fruit was accurately weighed and extracted with 25 ml of dichloromethane by refluxing in a water bath at 60 °C for 30 min, cooled and filtered. The filtrate was discarded and the residue extracted with 25 ml of 80 % ethanol (adjust to pH 1,0 with hydrochloride acid) by refluxing in a water bath at 80 °C for 30 min, cooled and filtered. The filtrate was evaporated to dryness, and the residue was dissolved with 2 ml aqueous ethanol (80 % volume fraction). The supernatant was packed into an aluminium oxide column ($OH^-$ form, a glass tube 10 mm to 15 mm in internal diameter and 20 cm in length, packed 10 cm with aluminium oxide) and eluted with 40 ml ethanol containing 5 % ammonium hydroxide. Subsequently, the eluted solution was evaporated to dryness and then dissolved with 10 ml ethanol. The supernatant was then filtered through a 0,22 µm Millipore filter unit prior to the HPLC analysis.

## D.3 Chromatographic system

**D.3.1 Column.**

**D.3.1.1 Stationary phase:** HILIC dihydroxypropyl group bonded to porous silica particles, 5 µm in diameter as analysing column or equivalent.

**D.3.1.2 Size:** $l$ = 250 mm, $\Phi$ = 4,6 mm.

**D.3.2 Mobile phase.**

**D.3.2.1 Mobile phase A:** water for chromatography R.

**D.3.2.2 Mobile phase B:** acetonitrile for chromatography R.

**D.3.2.3 Isocratic elution:** a mixture of mobile phases A and B (19:81).

**D.3.3 Flow rate:** 0,7 ml/min.

**D.3.4 Detector:** 195 nm.

**D.3.5 Column temperature:** 30 °C.

**D.3.6 Injection volume:** 10 µl.

## D.4 Content calculation of betaine

**D.4.1** The content of betaine, $C_{bet}$ (%) is calculated with Formula (D.1).

$$C_{bet} = \frac{c_s \times 10^{-3} \times 100}{M \times (1 - C_m)} \times 100\,\% \quad \text{(D.1)}$$

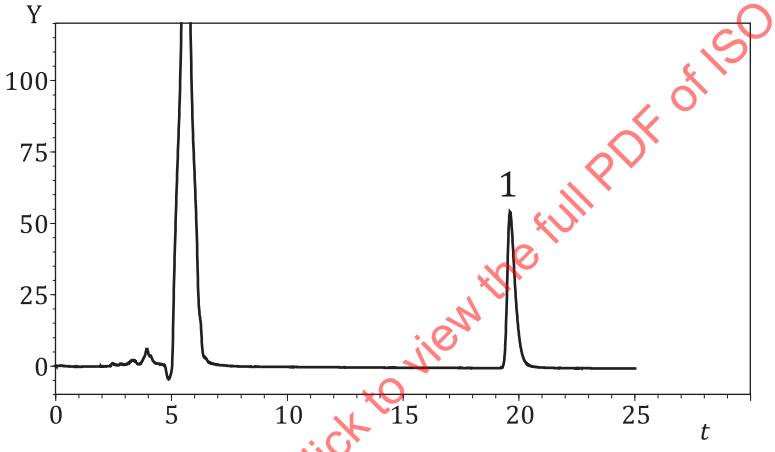
where

$C_s$ is the average content of the sample (mg/ml);

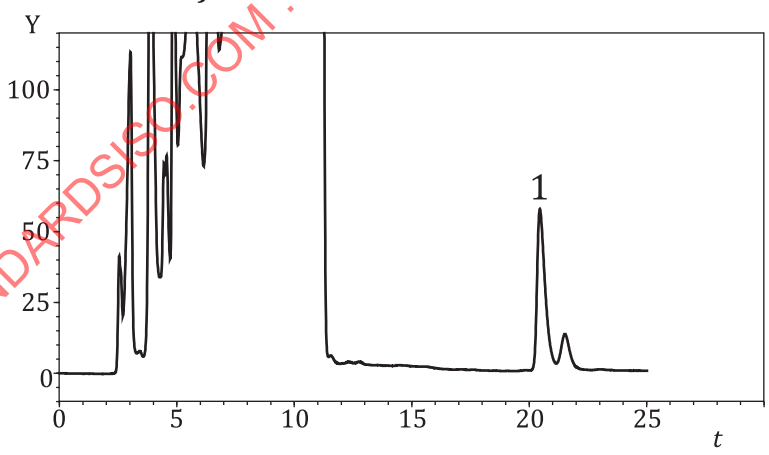
$M$ is the mass of *Lycium barbarum* and *Lycium chinense* fruit taken to prepare the sample solution (g);

$C_m$ is the moisture content of the sample (%).

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




**a) Betaine reference standard**




**b) *Lycium barbarum* fruit**