International Standard

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Traditional Chinese medicine — *Eucommia ulmoides* stem bark

*Médecine traditionnelle chinoise — Écorce d'*Eucommia ulmoides

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Foreword

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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.

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Introduction

Eucommia ulmoides stem bark has the effects of nourishing the liver and kidneys and strengthening bones. It is recorded in *Compendium of Materia Medica* and *Sheng Nong's herbal classic*, and is clinically used in the treatment of hypertension, chronic kidney disease, arthritis and other diseases. Various commodity specifications of *Eucommia ulmoides* stem bark, including its medicinal materials, decoction pieces and extracts, are widely traded in China, the Republic of Korea, Japan, Malaysia, Vietnam, Singapore, Thailand, Canada and the United States of America. Although *Eucommia ulmoides* stem bark has been included in several national pharmacopoeias and regional standards, the requirements differ. In addition, there are many problems with *Eucommia ulmoides* stem bark products on the market, such as mixed use of non-medicinal parts and irregular processing, which can lead to a decline in the quality of this medicinal herb. Therefore, it is necessary to establish a unified International Standard to guarantee the quality and safety of *Eucommia ulmoides* stem bark.

As national implementation can 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 — *Eucommia ulmoides* stem bark

1 Scope

This document specifies the minimum requirements and test methods for *Eucommia ulmoides* stem bark.

It is applicable to *Eucommia ulmoides* stem bark that is sold and used as natural medicines in international trade, including Chinese materia medica (whole medicinal materials) and raw decoction pieces.

It is not applicable to processed decoction pieces of *Eucommia ulmoides* stem bark.

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
***Eucommia ulmoides* stem bark**
outer covering of the stem of *Eucommia ulmoides* Oliv.

3.2
reference medicine
authentic medicine from *Eucommia ulmoides stem bark* (3.1) used for reference in thin-layer chromatographic analyses of the sample

3.3
batch
samples collected from the same particular place at the same time

[SOURCE: ISO 21317:2019, 3.5]

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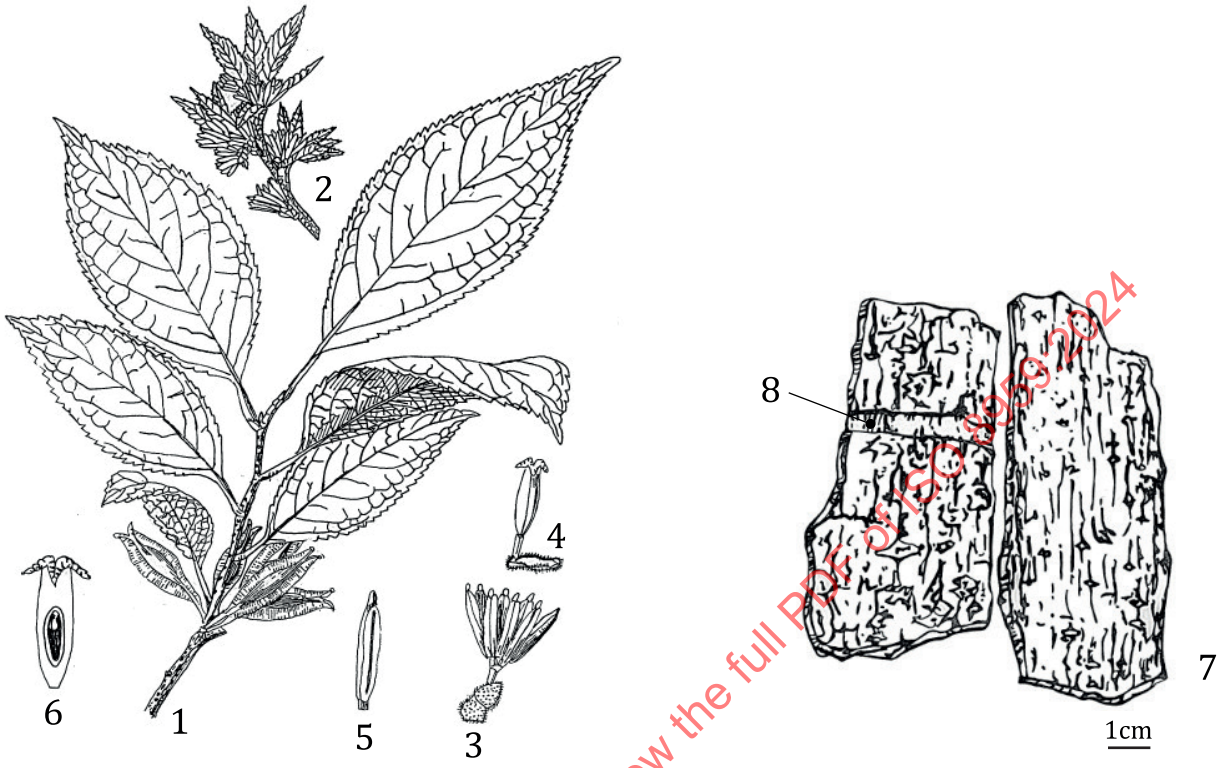
4 Descriptions

Eucommia ulmoides stem bark is the dried stem bark of *Eucommia ulmoides* Oliv. in the family of Eucommiaceae, as shown in Figure 1.



Key

1 fruiting branch
2 flowering branch
3 male flower
4 female flower
5 stamen lateral view
6 ovary longitudinal section
7 stem bark
8 rubber thread

Figure 1 — Structure of *Eucommia ulmoides* stem bark

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5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Eucommia ulmoides* stem bark shall be clean and dry.

b) The presence of living insects, mouldy root and external contaminants which are visible to the naked eye shall not be permitted.

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5.2 Macroscopic characteristics

They are in flat pieces or the two edges somewhat curved inward, varying in size and 0,3 cm to 0,7 cm thick. The outer surface is pale brown or greyish-brown, markedly wrinkled or fissured and channelled longitudinally. Some pieces are relatively thin, showing distinct lenticels when the coarse bark is unscraped. The inner surface is dark purple and smooth. The texture is fragile, easily broken, fracture linked by fine, dense, silvery, elastic and resinous threads. The odour is slight and the taste is slightly bitter.

5.3 Microscopic characteristics

5.3.1 Transverse section

The transverse section sometimes shows evidence of rhytidome outside the cork cells. Cork consists of several rows of cells. Phloem consists of five to seven bands of lignified stone cells; each band contains two to six rows of stone cells, with fibres nearby. Phloem rays are two to three cells wide. Resinous masses are scattered.

5.3.2 Powder

The colour is brown. The resinous threads are stripe-shaped or twisted into masses, the surface granular. Sclereids are numerous, mostly in groups, subrectangular to subrounded, elongated-rectangular or irregular, 20 µm to 80 µm in diameter, up to 180 µm long and thick-walled, with some containing resinous masses. Cork cells are polygonal in surface view, 15 µm to 40 µm in diameter, with unevenly thickened, lignified and finely pitted walls; rectangular in lateral view, walls thicker on three sides and relatively thin on one side, pit canals distinct. See Figure 2.

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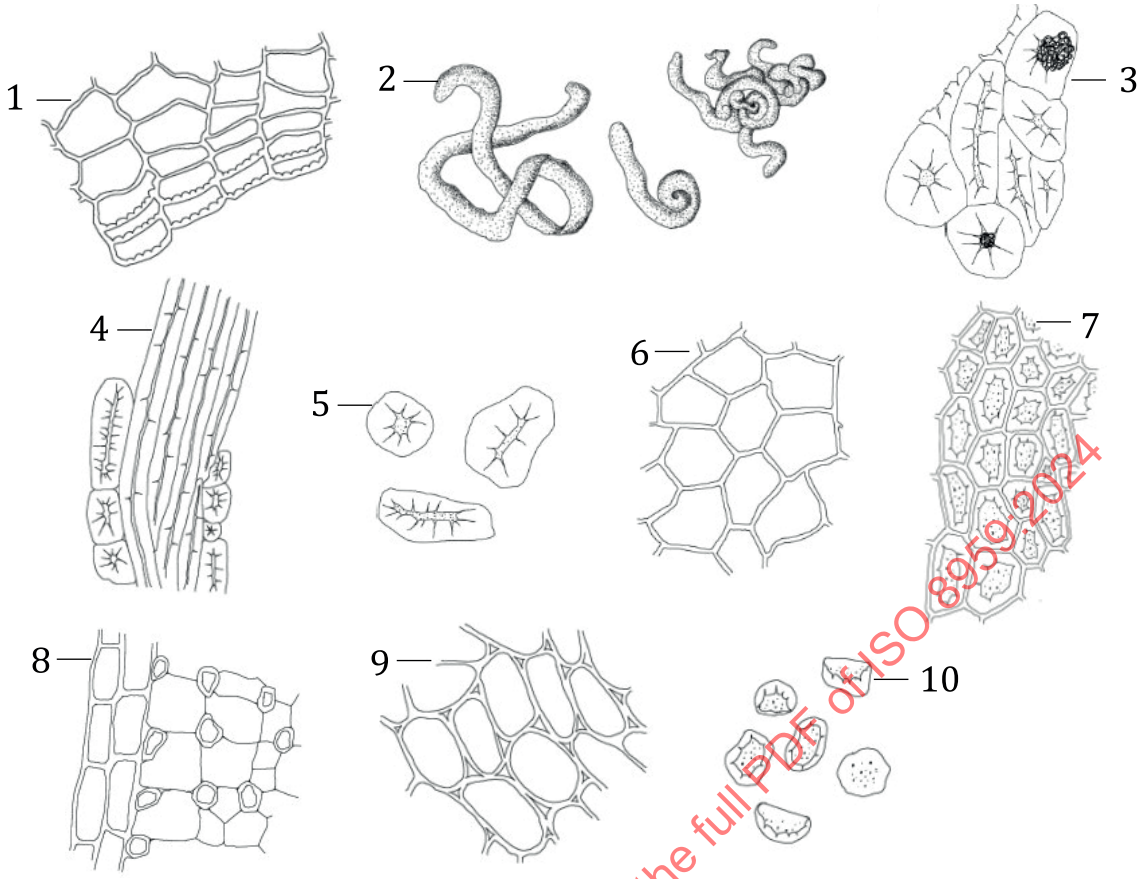

Key

1 cork consisting of layers of cells
2 ribbon-shaped latex fragments
3 sclereids in groups
4 fibres in groups associated with sclereids
5 isolated sclereids
6 cork consisting of polygonal cells
7 hard cork consisting of polygonal cells
8 rare fragments of phloem parenchyma
9 ovoid parenchyma cells
10 isolated hard cork cells

Figure 2 — Microscopic characteristics of *Eucommia ulmoides* stem bark powder

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5.4 Moisture

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

5.5 Total ash

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

5.6 Acid-insoluble ash

The mass fraction of acid insoluble ash should be determined.

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5.7 Thin-layer chromatogram

When TLC identification is performed, the chromatogram of sample solution shall present the spots with the same colour and positions corresponding to those from the reference medicine solution.

5.8 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extracts should be determined.

5.9 Content of marker compound(s)

The mass fraction of marker compound(s) such as pinoresinol diglucoside should be determined.

5.10 Heavy metals

The mass fractions of heavy metals (elemental impurities) such as arsenic, mercury, lead and cadmium should be determined.

5.11 Pesticide residues

The mass fractions of pesticide residues such as benzene hexachloride, DDT and pentachloronitrobenzene should be determined.

5.12 Sulfur dioxide residues

The mass fraction of sulfur dioxide residues should be determined.

6 Sampling

Sampling of *Eucommia ulmoides* stem bark shall be carried out in accordance with ISO 23723.

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 and by smell.

7.2 Determination of moisture

The test method specified in ISO 23723 shall apply.

7.3 Determination of total ash

The test method specified in ISO 23723 shall apply.

7.4 Determination of acid-insoluble ash

The test method specified in ISO 23723 shall apply.

7.5 Thin-layer chromatographic identification

See Annex A for additional information.

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7.6 Determination of ethanol-soluble extractives

The test method specified in ISO 23723 shall apply.

7.7 Determination of marker compound(s)

See Annex B for additional information.

7.8 Determination of heavy metals

The test method specified in ISO 18664 shall apply.

7.9 Determination of pesticide residues

The test methods specified in ISO 22258 shall apply.

7.10 Sulfur dioxide residues

The test method specified in ISO 22590 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 used, with reference to this document;

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 or 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 can damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The storage condition specified in ISO 22217 shall apply.

The product 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 labelled on the packages in accordance with the method specified in ISO 21371:

a) product name;

b) category of the product in the marketed country or region;

c) net mass or quantity;

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d) contact information;

e) name of raw materials;

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

g) storage method;

h) batch or lot number.

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Annex A
(informative)

Thin-layer chromatographic (TLC) identification

A.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

a) water: distilled water;

b) methanol, n-butanol, dichloromethane, trichloromethane: analytical grade;

c) developing solvent system: trichloromethane, methanol and water in a volume ratio of (10:5:1);

d) spray reagent: 20 % dilute sulfuric acid;

e) prefabricated thin-layer high-efficiency silica gel F_{254} or equivalent plate: 100×200 mm high-efficiency silica gel plate, activated at 105 °C for half an hour before use.

A.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

a) sieve: sieve hole diameter (average): 850 µm ± 29 µm, 24 mesh;

b) conical flask: 500 ml;

c) analytical balance: weighing accuracy 0,01 mg;

d) constant temperature blast oven: variable temperature range 30 °C to 300 °C, temperature control accuracy of ±1 °C.

A.3 Sample analysis

A.3.1 Preparation of the test solution

Weigh 2,5 g of the powdered sample and place it in a 100 ml conical flask, then add 30 ml of methanol. Sonicate the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 20 ml of water. Transfer the aqueous solution to a separatory funnel. Extract the solution with 50 ml of dichloromethane and discard the dichloromethane layer. Extract the aqueous layer with 50 ml of n-butanol. Evaporate the n-butanol extract to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 ml of methanol.

A.3.2 Reference solution

Dissolve 1 mg of pinoresinol diglucoside and 1 mg syringaresinol di-O-glucoside in 1 ml of methanol.

A.3.3 Reference medicine

Weigh approximately 2,5 g of the reference medicine and prepare the reference medicine by the same method as described under test solution.

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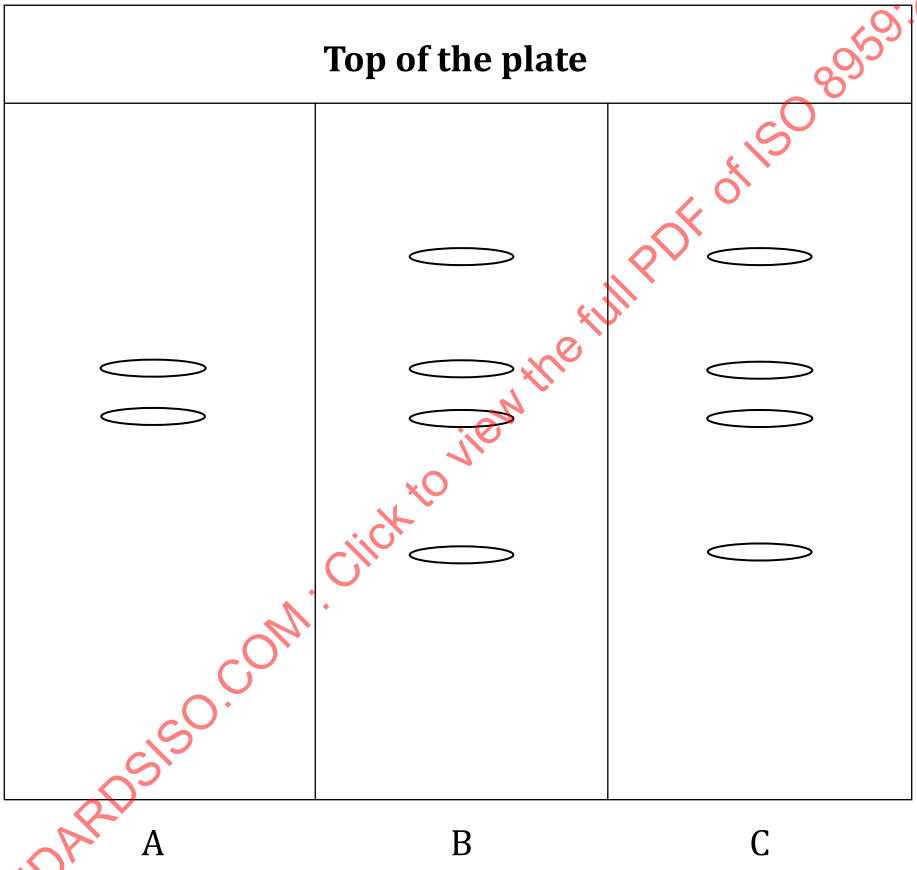
A.3.4 Identification by TLC

Carry out the method by using a high-efficiency silica gel F_{254} plate and a freshly prepared developing solvent system as described in A.1. Apply standard solution (5 µl), reference medicine solution (5 µl) and the test solution (5 µl) to the plate. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105 °C until the spots or bands become visible (about 10 min). Examine the plate under visible light.

A.4 Record the result

In the same high-efficiency silica gel F_{254} plate, the chromatogram of the test solution corresponds to the chromatogram of the reference standard solution and reference medicine, and spots of the same colour are displayed.

Typical reference TLC chromatograms are shown in Figure A.1.



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Key

A reference standard solution pinoresinol diglucoside and syringaresinol di-O-glucoside (from top to bottom)

B reference medicine

C test solution

Figure A.1 — Schematic diagram of typical TLC chromatogram of *Eucommia ulmoides* stem bark

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Annex B
(informative)

Determination of marker compound(s) by HPLC-UV

B.1 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

a) water: analytical grade;

b) methanol: HPLC grade;

c) pinoresinol diglucoside reference substance: for content analysis, purity ≥ 98 %.

B.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

a) centrifuge tube: 50 ml;

b) analytical balance: weighing accuracy 0,01 mg;

c) ultrasonicator: power 300 W, frequency 40 kHz;

d) HPLC-UV system: UV.

B.3 Preparation of reference standard solution

Dissolve reference substance of pinoresinol diglucoside CRS in methanol to prepare the reference standard solution of 1,0 mg/ml. Store at −20 °C if necessary.

B.4 Preparation of the test solution

Weigh approximately 2,0 g of the powder into a 100 ml centrifuge tube. Add 60 ml 20 % methanol and sonicate for 30 min (with the power 200 W, frequency 40 kHz), then add 20 % methanol to make up the weight loss. Filter through a 0,45-μm nylon filter, then take the filtrate as the test solution.

B.5 Chromatographic system

B.5.1 Column

B.5.1.1 Stationary phase: octadecylsilane bonded silica gel (5 μm) or equivalent for analysing.

B.5.1.2 Size: l = 0,20 m, $Ø$ = 4,6 mm.

B.5.2 Mobile phase

B.5.2.1 Mobile phase A: methanol.

B.5.2.2 Mobile phase B: water containing 0,05 % phosphoric acid.

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B.5.3 Gradient elution

Table B.1 — Mixture of mobile phases A and B in gradient elution

Time min	Mobile phase A %	Mobile phase B %
0 to 40	5→15	95→85
40 to 50	15→20	85→80
50 to 55	20→25	80→75
55 to 60	25→40	75→60

B.5.4 Flow rate: 0,8 ml/min.

B.5.5 Detector: 230 nm.

B.5.6 Injection volume: 10 µl.

The number of theoretical plates calculated by pinoresinol diglucoside peak should not be less than 1 000.

B.6 Determination

Inject 10 µl each of the reference solution and the test solution into the column and determine the HPLC chromatograms of *Eucommia ulmoides* stem bark, see Figure B.1. With the peak area of pinoresinol diglucoside CRS as an internal reference, calculate the contents of geniposidic acid, asperuloside, syringin, geniposide and syringaresinol di-O-glucoside. Within a certain linear range, the amount of a component is directly proportional to the response value of the detector. Taking pinoresinol diglucoside CRS as the internal reference, establish the relative correction factor between the internal reference and other components, and calculate the content of other components through the relative correction factor (Table B.1). The relative correction factor is calculated with Formula (B.1):

$$f_{s/i} = f_s/f_i = A_s \times C_i / (A_i \times C_s) \quad \text{(B.1)}$$

where

$f_{s/i}$ is the relative correction factor;

f_s is the correction factor of pinoresinol diglucoside CRS;

f_i is the correction factor of the test compound;

A_s is the peak area of pinoresinol diglucoside CRS;

A_i is the peak area of the test compound;

C_s is the concentration of pinoresinol diglucoside CRS;

C_i is the concentration of the test compound.

Confirm the five peak locations by relative retention time for five compounds in Table B.2. The retention time of test compound peak of the sample, f, is calculated with Formula (B.2):

$$f = R_{Tx} / R_{Ts} \quad \text{(B.2)}$$

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where

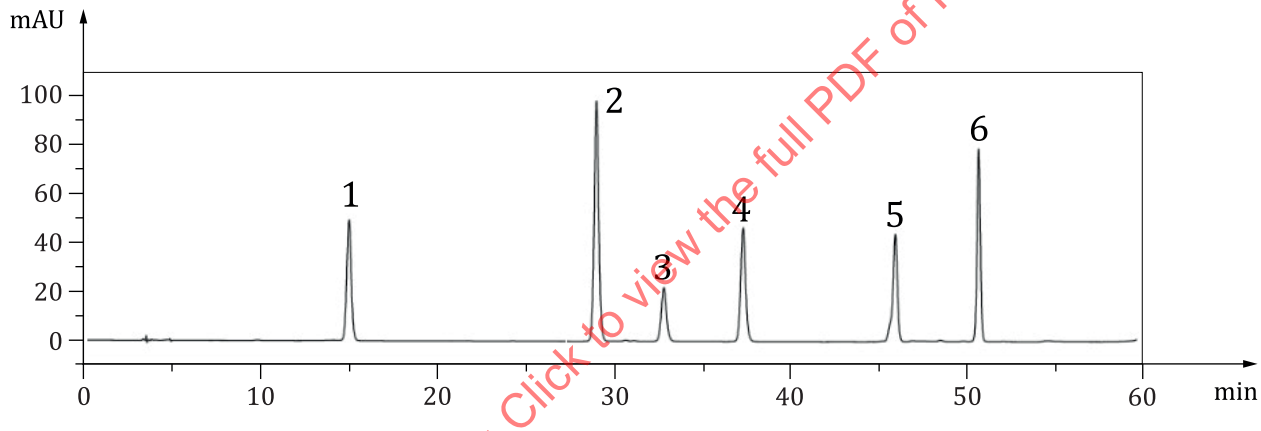
R_{Tx} is the retention time of the test compound;

R_{Ts} is the retention time of pinoresinol diglucoside CRS.

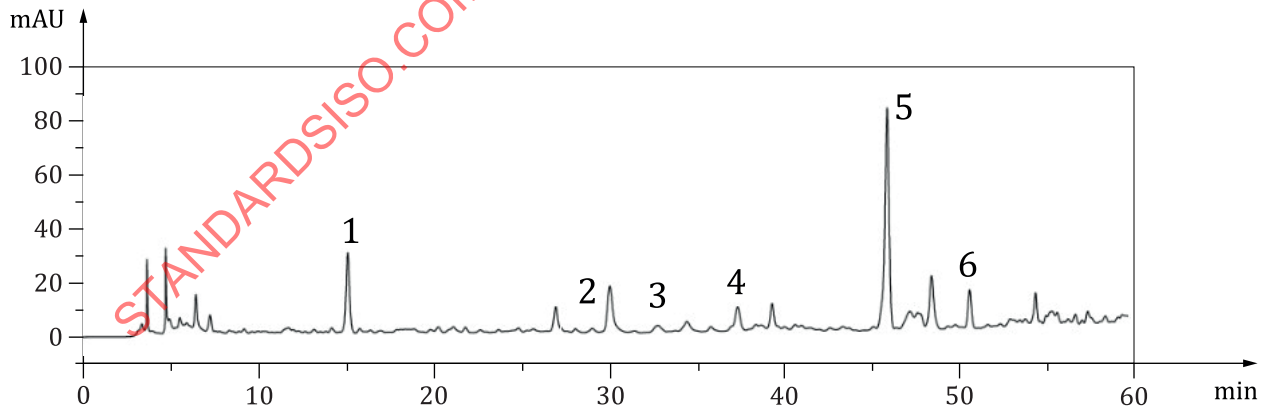
Table B.2 — The relative correction factor and relative retention time for six compounds

Test compound peak	Relative correction factor	Relative retention time
geniposidic acid	1,14	0,33
asperuloside	0,51	0,63
syringin	2,01	0,71
geniposide	1,00	0,81
pinoresinol diglucoside	1,00	1,00
syringaresinol di-O-glucoside	0,85	1,10

The relative correction factor and relative retention time of the test peak should remain within ±5 % of the value as described in Table B.2. Validate the methodology for accuracy, precision and repeatability, if necessary.



a) Chromatogram of reference standard solution



b) Chromatogram of test solution

Key

1 geniposidic acid
2 asperuloside
3 syringin

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4 geniposide
5 pinoresinol diglucoside
6 syringaresinol di-O-glucoside

Figure B.1 — HPLC chromatograms of *Eucommia ulmoides* stem bark

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