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Traditional Chinese medicine — *Lonicera japonica* flower

Médecine traditionnelle chinoise — Fleur de Lonicera japonica

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

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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

Lonicera japonica flower is the dried flower bud or flower starting to bloom of *Lonicera japonica* Thunb., which is recorded in the Chinese, South Korean, Japanese and United States Pharmacopeias. *Lonicera japonica* flower is internationally recognized as a traditional Chinese herbal medicine, and there is great demand for it in the international market. However, there are many problems seriously affecting the international trade of *Lonicera japonica* flower, including the following.

1) Quality requirements for *Lonicera japonica* flower are different among different countries and regions.

2) *Lonicera japonica* flower is often substituted with fake and inferior versions.

3) Different collecting times, processing methods, packaging, transportation and storage conditions often result in different qualities of *Lonicera japonica* flower.

Therefore, the establishment of an international standard for *Lonicera japonica* flower is necessary to guarantee the quality, safety and consistency of this valuable herbal medicine. This document includes sections on morphology evaluation, physicochemical indexes and heavy metals content.

As national implementation may differ, National Standards Bodies are invited to modify the values given in 5.4, 5.5, 5.6, 5.7, 5.9 and 5.10 in their national standards. Examples of national and regional values are given in Annex D.

Traditional Chinese medicine — *Lonicera japonica* flower

1 Scope

This document specifies minimum requirements and test methods for *Lonicera japonica* flower, which is derived from the plant *Lonicera japonica* Thunb. It is applicable to *Lonicera japonica* flower that is sold and used as traditional Chinese medicine.

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 1666, *Starch — Determination of moisture content — Oven-drying method*

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

World Health Organization. 2011, *Quality control methods for herbal materials, General advice on sampling*

CODEX STAN 1: 1985, *Codex general standard for the labeling of prepackaged foods*

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
***Lonicera japonica* flower**
dried flower bud or flower starting to bloom of *Lonicera japonica* Thunb.

3.2
chlorogenic acid content
mass fraction of chlorogenic acid in the sample determined in accordance with Annex B

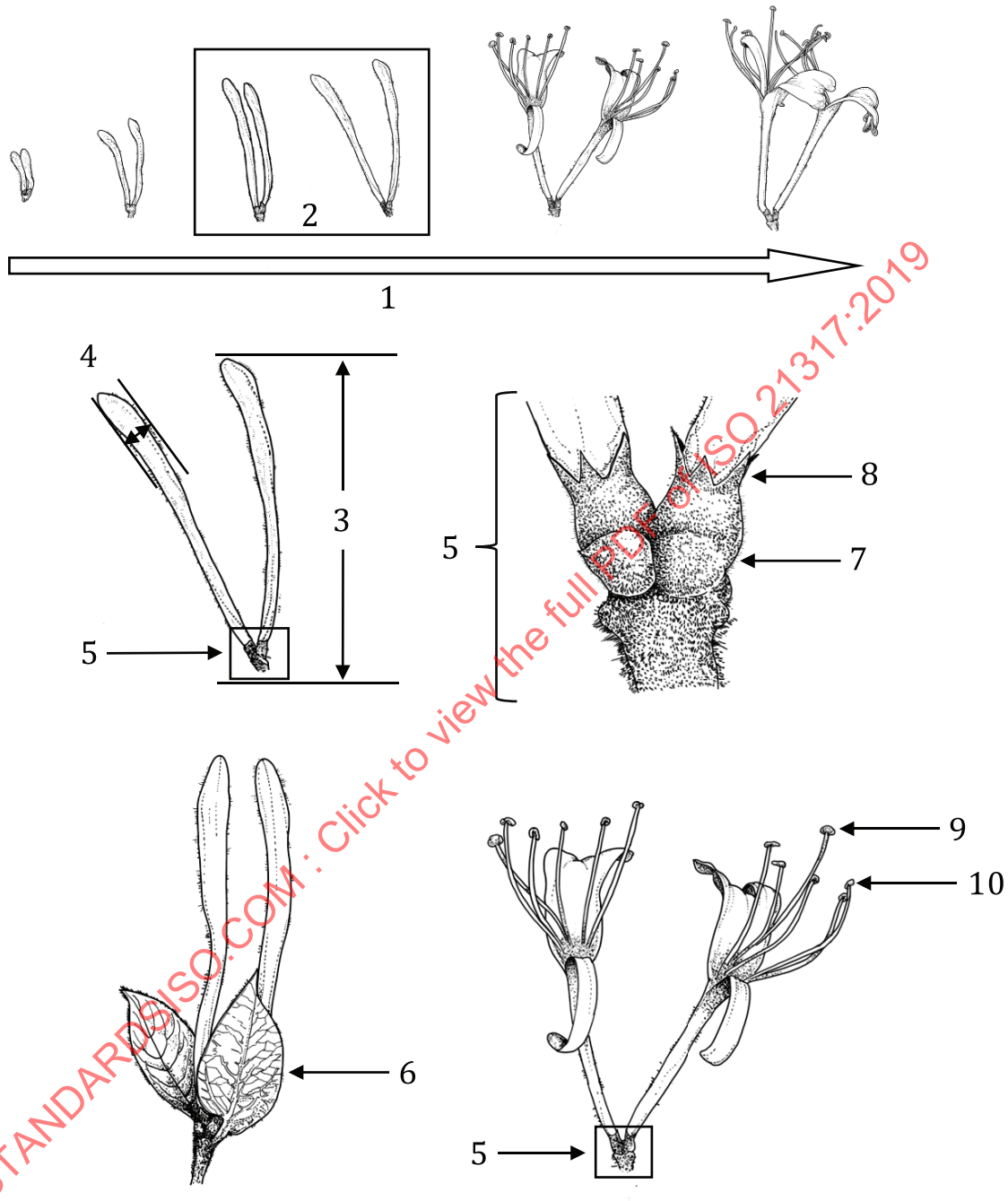
3.3
luteoloside content
mass fraction of luteoloside in the sample determined in accordance with Annex C

3.4
dilute ethanol-soluble extract
mass fraction of extract obtained from the sample using the method specified in 7.6

3.5
batch
samples collected from the same particular place at the same time

4 Descriptions

In this document, *Lonicera japonica* flower is the dried flower bud or flower starting to bloom of *Lonicera japonica* Thunb. It is externally yellowish-white, greenish-white to yellowish-green, gradually darkening over time to a golden colour (see Figure 1).



Key

1	*Lonicera japonica* flower in different flowering stages	6	bract (leaf-like)
2	*Lonicera japonica* flower selected for medicinal use	7	calyx tube
3	length	8	calyx lobes
4	diameter	9	pistil
5	calyx	10	stamen

Figure 1 — The structure of *Lonicera japonica* flower

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling.

a) *Lonicera japonica* flower shall be the dried flower bud or flower starting to bloom.

b) *Lonicera japonica* flower shall be clean and free from foreign matter except for a few leaf-like bracts.

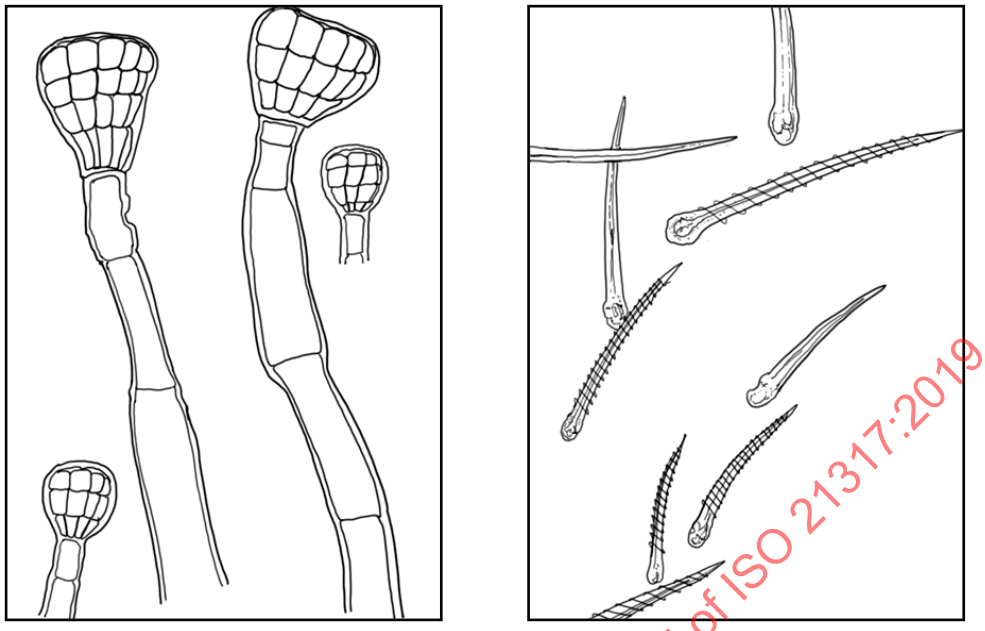
c) The presence of living insects, mould and external contaminants which are visible to the naked eye shall not be permitted.

5.2 Macroscopic characteristics

Flower buds are clavate, tapered downwards, slightly curved; 1,5 cm to 3,5 cm long, about 3 mm in diameter in the upper part and 1,5 mm in diameter in the lower part, with a densely pubescent surface; externally yellowish-white or greenish-white, gradually darkening over time to yellowish-brown. The foliaceous bracts are occasionally visible. The calyx is green, pubescent, five-lobed at the apex and about 2 mm long.

5.3 Microscopic characteristics

Lonicera japonica flower pedicels are covered with numerous glandular hairs and non-glandular hairs. The heads of glandular hairs are multicellular, turbinate, subround or slightly oblate, usually 30 µm to 70 µm in diameter, exceptionally up to 110 µm. The stalks of glandular hairs are unicellular or multicellular with up to five cells, usually 20 µm to 70 µm long, exceptionally up to 700 µm. There are two types of non-glandular hairs: (i) thick walls, unicellular, 45 µm to 900 µm long, 15 µm to 40 µm in diameter, with fine verrucae on the surface, some have a corneous spiral; (ii) thin walls, slender, curved or shrinkage, with fine verrucae on the surface (see Figure 2).



1 2

Key

1 glandular hairs
2 non-glandular hairs

Figure 2 — The structures of glandular hairs and non-glandular hairs

5.4 Moisture

The mass fraction of moisture should not be more than 15,0 %. See the values listed in Annex D, Table D.1 for additional information.

5.5 Total ash

The mass fraction of total ash should not be more than 10,0 %. See the values listed in Annex D, Table D.1 for additional information.

5.6 Acid-insoluble ash

The mass fraction of acid-insoluble ash should not be more than 3,0 %. See the values listed in Annex D, Table D.1 for additional information.

5.7 Dilute ethanol-soluble extract

The mass fraction of dilute ethanol-soluble extract should not be less than 16,0 %. See the values listed in Annex D, Table D.2 for additional information.

5.8 Identification of chlorogenic acid and luteoloside

The identification of chlorogenic acid and luteoloside with thin layer chromatography (TLC) shall present spots obtained from the test and reference solutions in the same positions with the same retention values and colour after evenly spraying the chromogenic agent.

5.9 Chlorogenic acid content

The mass fraction of chlorogenic acid should not be less than 1,5 %. See the values listed in Annex D, Table D.2 for additional information.

5.10 Luteoloside content

The mass fraction of luteoloside should not be less than 0,05 %. See the values listed in Annex D, Table D.2 for additional information.

5.11 Heavy metals content

Heavy metals including lead, arsenic, cadmium and mercury shall be determined. See the values listed in Annex D, Table D.3 for additional information.

6 Sampling

Sampling of *Lonicera japonica* flower shall be in accordance with the World Health Organization 2011 *Quality Control Methods for Herbal Materials*, *General Advice on Sampling*.

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

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

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 60 units — i.e. take samples from six packages.

d) From each selected container or package, 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 carefully mixed.

e) The average sample is obtained by quartering. Take some of the pooled sample, adequately mixed, place in an even, 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 are tested for the measurement and analyses specified in Table 1. Ground samples shall be used in the measurement and analyses, except the identification of macroscopic characteristics. The test samples shall be finely ground into 65 mesh, carefully mixed and sealed.

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

Maximum weight of batch kg	Minimum weight of final sample g		
	For analysis of chlorogenic acid	For analysis of luteoloside	For other analyses
5 000	250	250	250
NOTE Other analyses include the identification of macroscopic and microscopic characteristics, the determination of moisture content, total ash, acid-insoluble ash, dilute ethanol-soluble extract and heavy metals content, and the identification of chlorogenic acid and luteoloside with TLC.			

7 Test methods

7.1 Macroscopic identification

Test samples of not less than 200 g shall be observed with the naked eye, and the length and diameter of the dried flowers measured.

7.2 Microscopic identification

Place about 1,0 mg of the powdered sample on a glass slide, add two or three drops of chloral hydrate solution and heat to make the sample object clear while stirring with a small glass rod to prevent boiling. After cooling, add one drop of diluted glycerin, put a cover glass on it, then observe the microscopic characteristics using an optical microscope.

7.3 Determination of moisture content

The testing method specified in ISO 1666 applies.

7.4 Determination of total ash content

The testing method specified in ISO 1575 applies.

7.5 Determination of acid-insoluble ash content

The testing method specified in ISO 1577 applies.

7.6 Determination of dilute ethanol-soluble extract content

Use a solution with a volume fraction of 50 % ethanol as dilute ethanol solvent. Take about 2 g (accurate to 0,01 g) of the sample, extract with 70 ml of dilute ethanol in a suitable flask, shaking occasionally for 5 h, and allow to stand for 16 h. Filter then wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 ml. Evaporate a 50 ml aliquot of the filtrate to dryness on a water bath, then dry at 105 °C for 4 h and cool in a desiccator (silica gel). Weigh the amount accurately then multiply it by two to obtain the amount of dilute ethanol-soluble extract. Calculate the percentage content of the extract in the sample on the dried basis.

7.7 Identification of chlorogenic acid and luteoloside

See Annex A for additional information.

7.8 Determination of chlorogenic acid content

See Annex B for additional information.

7.9 Determination of luteoloside content

See Annex C for additional information.

7.10 Determination of heavy metals content

The testing method specified in ISO 18664 applies.

8 Test report

For each test, 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 could 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

Lonicera japonica flower shall be packaged with moisture-proof packaging. The packaging shall not transmit any odour or flavour to the product and shall not contain substances which could damage the product or constitute a health risk.

Lonicera japonica flower shall be sealed then stored in a clean, dry, shady, cool and well ventilated place. The storage temperature for *Lonicera japonica* flower shall not be over 25 °C. The relative humidity shall not be over 65 %. The storage time shall not exceed 24 months.

Lonicera japonica flower shall be protected from light, moisture, pollution and entry of foreign matter during long-distance delivery.

10 Marking and labelling

Refer to the method specified in the CODEX STAN 1:1985, *Codex general standard for the labeling of prepackaged foods*. The following items shall be marked or labelled on the packages:

a) the product name and Latin scientific name of the original plant;

b) all quality features indicated in 5.2 to 5.11, determined in accordance with the methods specified in Clause 7;

c) the maximum weight of the batch and the minimum weight of samples specified in Table 1;

d) the country and province/state of origin of the product, as well as the name, trademark or logo of the producer and supplier;

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

f) the storage method;

g) items required by regulatory body of the destination country.

Annex A
(informative)

Identification of chlorogenic acid and luteoloside

A.1 Preparation of test solution

Put 1 g of the test sample powder into a flask, add 5 ml of 70 % ethanol-water solution and ultrasound for 30 min. Filter, and use the liquid as the sample solution.

A.2 Preparation of reference solutions

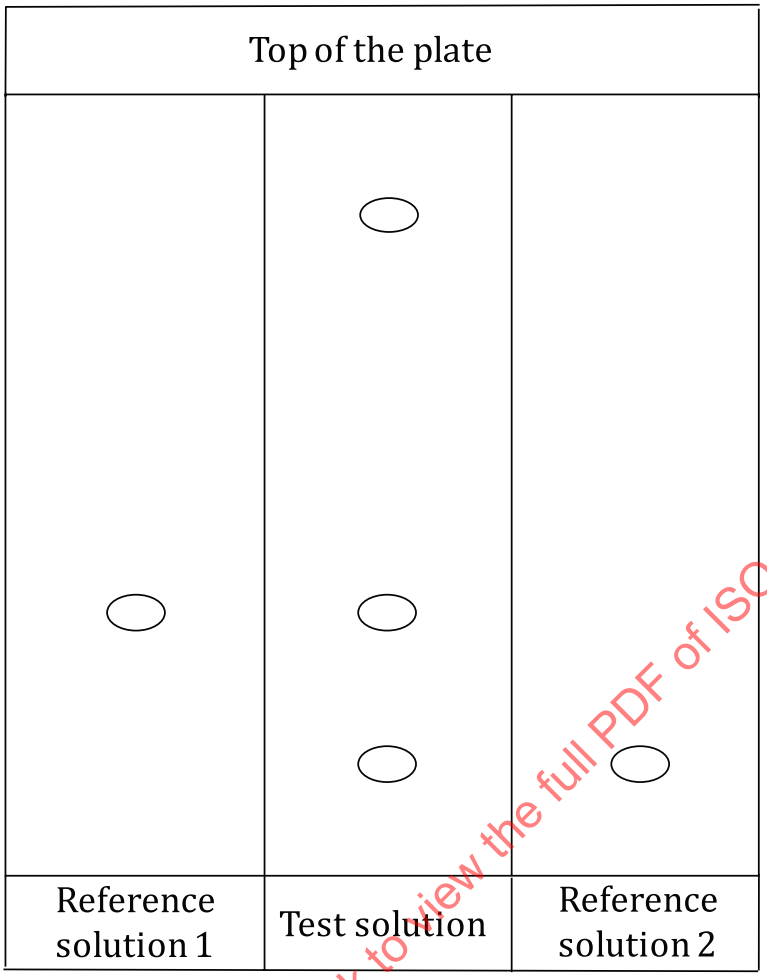
Dissolve 1 mg of reference substance chlorogenic acid and luteoloside in 1 ml of 70 % ethanol-water solution separately to obtain the reference solutions.

A.3 Preparation of chromogenic agent

Dissolve 2 g of ferric chloride in 100 ml of ethanol to obtain the chromogenic agent.

A.4 Identification

Spot 10 µl of each of the three solutions in A.1 to A.3 on the same silica gel GF254 plate, previously dried in an oven at 105 °C for 30 min. Develop the plate with a solution of the mixture of ethyl acetate, acetone, formic acid and water (volume fractions 20:3:1,5:1,5). Remove the plate and dry in air. Evenly spray the chromogenic agent. Identify the chlorogenic acid and luteoloside spots of the test solution by comparing the positions (or retention values) and colours (a grey-black colour for chlorogenic acid spot and a grey-green colour for luteoloside spot) with those of reference solutions. Typical reference TLC chromatograms are shown in Figure A.1.



Key

Reference solution 1 luteoloside reference solution
Reference solution 2 chlorogenic acid reference solution
Test solution *Lonicera japonica* flower test solution

Figure A.1 — Schematic diagram of typical reference TLC chromatograms of *Lonicera japonica* flower

Annex B
(informative)

Determination of chlorogenic acid content

B.1 Preparation of reference solution

Dissolve an appropriate amount of reference substance chlorogenic acid in 50 % methanol-water solution to obtain a reference solution containing 0,04 mg of chlorogenic acid in each millilitre.

B.2 Preparation of test solution

Put about 0,5 g (accurate to 0,000 1 g) of the ground sample into a conical flask with a glass stopper. Accurately add 50 ml of 50 % methanol-water solution, weigh accurately and ultrasound for 30 min, then cool and weigh again. Replenish the loss of solvent with 50 % methanol-water solution, shake then filter. Transfer 5 ml of this solution to a volumetric flask and dilute to 25 ml with 50 % methanol-water solution as the test solution.

B.3 Chromatographic system

B.3.1 Column.

B.3.1.1 Stationary phase: octadecyl silane chemically bonded to porous silica particles, 5 μm in diameter as analysing column or equivalent.

B.3.1.2 Size: l = 0,25 m, $Ø$ = 4,6 mm.

B.3.2 Mobile phase: acetonitrile: 0,4 % phosphoric acid in water (13:87).

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

B.3.4 Detection wavelength: 327 nm.

B.3.5 Injection volume: 10 μl.

B.3.6 System suitability: number of theoretical plates: minimum 1 000, calculated for the peak due to chlorogenic acid in the chromatogram.

B.4 Detection and content calculation of chlorogenic acid

Accurately draw 10 μl of the reference solution and 10 μl of the test solution, perform detection under the conditions in B.3, and the typical reference HPLC chromatogram of *Lonicera japonica* flower is shown in Figure B.1. Obtain the peak area of chlorogenic acid using the automatic integration method.

The mass fraction of chlorogenic acid in *Lonicera japonica* flower, ^{w}C, expressed as a percentage by mass on a sample, is calculated using Formula (B.1) (on the dried basis):

$$^{w}C = \frac{A_{\text{sam}} \times m_{\text{ref}} \times V_{\text{sam}} \times p_{\text{ref}} \times 5 \times 100}{A_{\text{ref}} \times m_{\text{sam}} \times V_{\text{ref}} \times p_{\text{sam}} \times 1\,000} \quad \text{(B.1)}$$

where

A_{sam} is the peak area of chlorogenic acid in the test sample solution;

A_{ref} is the peak area of chlorogenic acid in the reference solution;

V_{sam} is the sample extraction volume, in millilitres;

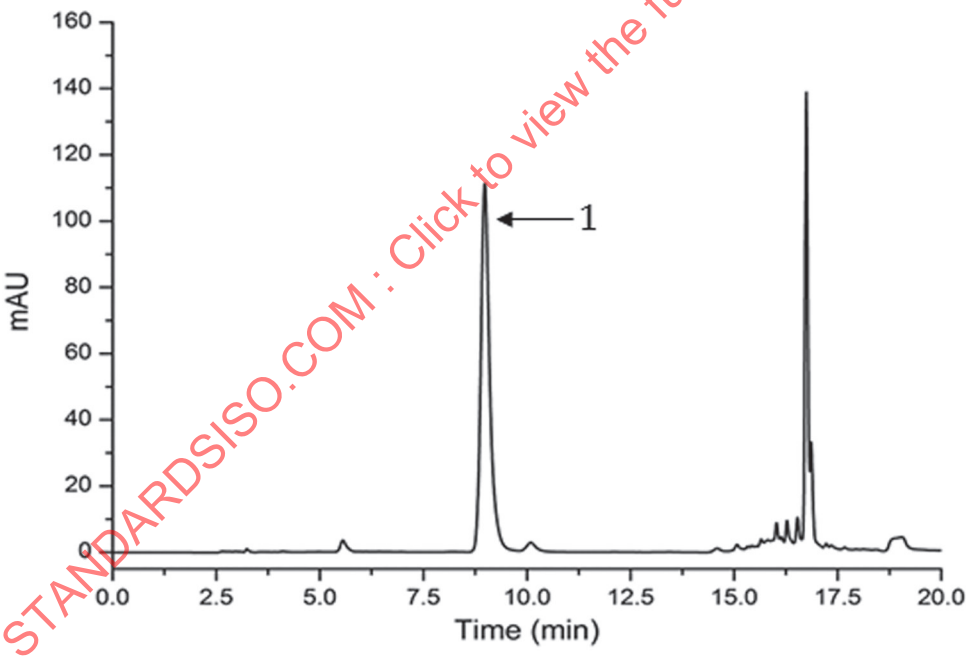
V_{ref} is the reference substance dissolved volume, in millilitres;

m_{sam} is the mass, in milligrams, of the sample test portion;

m_{ref} is the mass, in milligrams, of the reference substance;

p_{sam} is the percentage content of the dried test sample;

p_{ref} is the percentage content of the reference substance.



Key

1 chlorogenic acid

Figure B.1 — Typical reference HPLC chromatogram of *Lonicera japonica* flower

Annex C
(informative)

Determination of luteoloside content

C.1 Preparation of reference solution

Dissolve an appropriate amount of reference substance luteoloside in 70 % ethanol-water solution to obtain the reference solution containing 0,04 mg of luteoloside in each millilitre.

C.2 Preparation of test solution

Put about 2 g (accurate to 0,000 1 g) of the ground sample into a conical flask with a glass stopper. Accurately add 50 ml of 70 % ethanol-water solution, weigh accurately, ultrasound for 60 min, then cool and weigh again. Replenish the loss of solvent with 70 % ethanol-water solution, shake and filter. Transfer 10 ml of this solution to a sample bottle, dry with nitrogen, then dissolve and dilute to 5 ml with 70 % ethanol-water solution as the test solution.

C.3 Chromatographic system

C.3.1 Column.

C.3.1.1 Stationary phase: phenyl groups chemically bonded to porous silica particles, 5 µm in diameter as analysing column or equivalent.

C.3.1.2 Size: l = 0,25 m, $Ø$ = 4,6 mm.

C.3.2 Mobile phase.

C.3.2.1 Mobile phase A: Acetonitrile.

C.3.2.2 Mobile phase B: 0,5 % glacial acetic acid-water solution.

C.3.2.3 Program of gradient elution.

Time (min)	Mobile phase A (volume fraction, %)	Mobile phase B (volume fraction, %)
0 to 15	10 to 20	90 to 80
15 to 30	20	80
30 to 40	20 to 30	80 to 70

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

C.3.4 Detection wavelength: 350 nm.

C.3.5 Injection volume: 10 µl.

C.3.6 System suitability: Number of theoretical plates: minimum 20 000, calculated for the peak due to luteoloside in the chromatogram.

C.4 Detection and content calculation

Accurately draw 10 µl of the reference solution and 10 µL of the test solution, perform detection under the conditions in C.3, and the typical reference HPLC chromatogram of *Lonicera japonica* flower is shown in Figure C.1. Obtain the peak area of luteoloside using the automatic integration method. The mass fraction of luteoloside in *Lonicera japonica* flower, ^{w}C, expressed as a percentage by mass on a sample, is calculated using Formula (C.1) (on the dried basis):

$$^{w}C = \frac{A_{\text{sam}} \times m_{\text{ref}} \times V_{\text{sam}} \times p_{\text{ref}} \times 100}{A_{\text{ref}} \times m_{\text{sam}} \times V_{\text{ref}} \times p_{\text{sam}} \times 1\,000 \times 2} \quad \text{(C.1)}$$

where

A_{sam} is the peak area of luteoloside in the test sample solution;

A_{ref} is the peak area of luteoloside in the reference solution;

V_{sam} is the sample extraction volume, in millilitres;

V_{ref} is the reference substance dissolved volume, in millilitres;

m_{sam} is the mass, in milligrams, of the sample test portion;

m_{ref} is the mass, in milligrams, of the reference substance;

p_{sam} is the percentage content of the dried test sample;

p_{ref} is the percentage content of the reference substance.