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Traditional Chinese medicine — *Artemisia argyi* leaf

La médecine traditionnelle Chinoise — Artemisia argyi *feuille*

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Foreword

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

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Introduction

Mugwort leaf is a traditional herbal medicine. It is mainly used for the treatment of irregular menstruation, infertility, hematemesis, epistaxis, metrorrhagia and pruritus in China and some other countries. It is also the raw material for famous moxibustion therapy, many pharmaceutical preparations and health care products. Mugwort leaf originates from the three species of plants *Artemisia argyi* Lévl. et Vant., *Artemisia princeps* Pampanini or *Artemisia montana* Pampanini in different countries. The three original species mainly grow in China and Korea, and the second and third species also grow in Japan. Among them, the dried leaf of *Artemisia argyi* Lévl. et Vant. has been used most extensively in the world. This document is established mainly to control the quality of *Artemisia argyi* leaf for direct medicinal and pharmaceutical purpose.

For reference, the limit values of the relevant target substance and moisture, total ash and acid-insoluble ash have been provided in Annex D. In addition, factors affecting the quality of *Artemisia argyi* leaf also include pH and organic matters of soil, light, harvest time, as well as cultivation techniques and so on. These factors cannot be controlled by sample analysis. Therefore, relevant information is described in Annex E as a reference.

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Traditional Chinese medicine — *Artemisia argyi* leaf

1 Scope

This document specifies the minimum requirements and test methods of *Artemisia argyi* leaf for medicinal use. It is suitable for identification and quality control of this herbal 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 928, *Spices and condiments — Determination of total ash*

ISO 930, *Spices and condiments — Determination of acid-insoluble ash*

ISO 939, *Spices and condiments — Determination of moisture content — Entrainment method*

ISO 6571, *Spices, condiments and herbs — Determination of volatile oil content (hydrodistillation method)*

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 http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
***Artemisia argyi* leaf**
dried leaf of the plant *Artemisia argyi* Lévl. et Vant. which belongs to the family Asteraceae

3.2
reference medicine
authentic reference medicine from the dried leaf of *Artemisia argyi* Lévl. et Vant., used for reference in TLC analyses of the sample

3.3
volatile oil content
all the substances in the sample entrained by steam under the conditions specified in Annex B

Note 1 to entry: It is expressed in ml per 100 g of dry duct.

[SOURCE: ISO 6571:2008, 3.1, modified — "in the sample" has been inserted and the term "International Standard" has been replaced with "Annex B".]

3.4
1,8-cineole content
mass fraction of 1,8-cineole ($C_{10}H_{18}O$) in the sample determined in accordance with the method of Annex B

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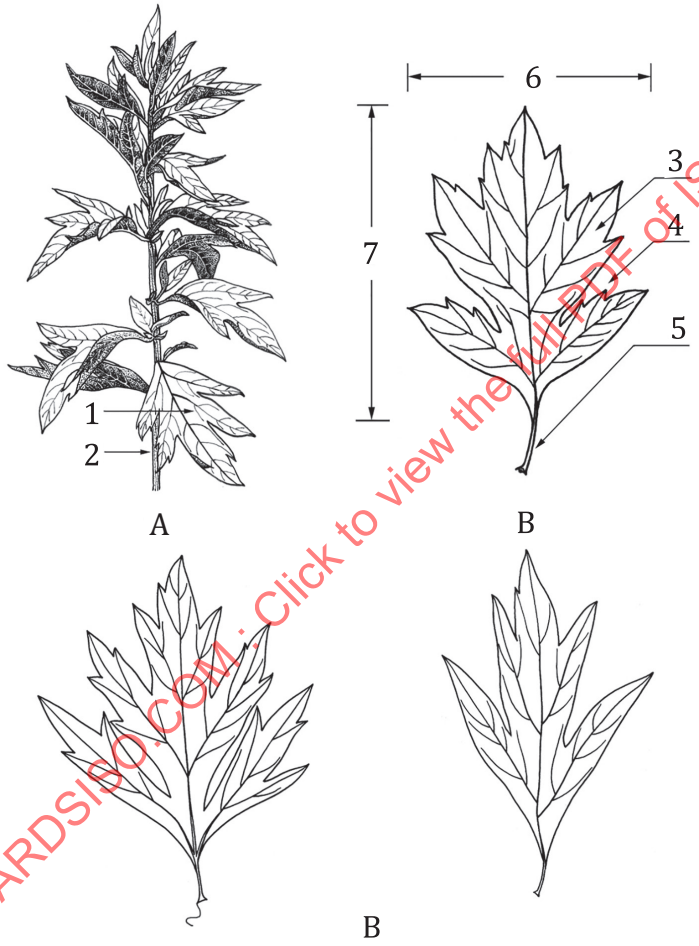
3.5
total flavonoids content
mass fraction of the total flavonoids in the sample determined in accordance with the method in Annex C

3.6
dilute ethanol-soluble extract
extract obtained from the sample determined in accordance with the method specified in 7.7

4 Description

In this document, *Artemisia argyi* leaf is the dried leaf from the plant *Artemisia argyi* Lévl. et Vant. (see Figure 1 A) and consists of leaf blade and petiole (see Figure 1 B).



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Key

A	plant of *Artemisia argyi* Lévl. et Vant.	4	dentate segment/wide sawtooth
B	leaves of the middle stem of *Artemisia argyi* Lévl. et Vant.	5	petiole
1	leaf blade	6	leaf blade width
2	stem	7	leaf blade length
3	segment		

Figure 1 — Plant of *Artemisia argyi* Lévl. et Vant. and structure of the leaf

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5 Requirements

5.1 General characteristics

The following requirements should be met before separating the bulk sample into test samples.

a) Artermisia argyi leaf shall be clean and free from foreign matter.

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

5.2 Macroscopic characteristics

The leaves are mostly crumpled or broken, with short petioles and usually without pseudostipule at the base. The leaf blade is broad ovate, rhombic-ovate, or ovate-elliptical, 5 cm to 9(16) cm long, 4 cm to 8(16) cm wide, and pinnatipartite to pinnatifid; segments in two or three pairs, elliptical-lanceolate, or elliptical-oblanceolate, frequently 1 cm to 1,5(3) cm wide, with margin irregularly dentate or lobed or wide serrate, dentate piece or wide sawtooth, mostly asymmetrically ovate-lanceolate, ovate-triangular, or entire. Smaller leaves are fewer, pinnatipartite, tri-cleft, and sometimes entire and elliptical to lanceolate. The upper surface is greyish-green or dark yellowish-green, possessing somewhat sparse pubescences, denser glandular dots and small pits; lower surface is covered densely with greyish-white tomenta. The petiole is usually 2 mm to 8 mm long, and up to 1,3 cm in the cultivated plants.

It has a specific, delicate aroma and slightly bitter and astringent taste.

5.3 Microscopic characteristics

The powder is greenish-brown. T-shaped hairs (A) are numerous, with a short uniseriate stalk consisting of two to six small cells, and perpendicularly capped by a relatively straight or undulating terminal cell (7 µm to 18 µm in diameter and up to 980 µm long) tapering at the ends. Soft hairs (B) are uniseriate, consisting of three to five cells with a very long and twisted apical cell, which are frequently broken. Glandular hairs (C), without stalk, consist of four or six oppositely-overlapped cells in lateral view (Ca) and in pairs and paramecium-like in apical view (Cb). Cluster crystals of calcium oxalate (D) are frequently 3 µm to 12 µm in diameter, mostly occurring in mesophyll cells. Fragments of epidermis in surface view (E) are with anomocytic stomas. Fragments of spiral vessels (F) can be frequently seen; vessels with bordered pit (G) and reticulate vessels (H) are sometimes visible (see Figure 2).

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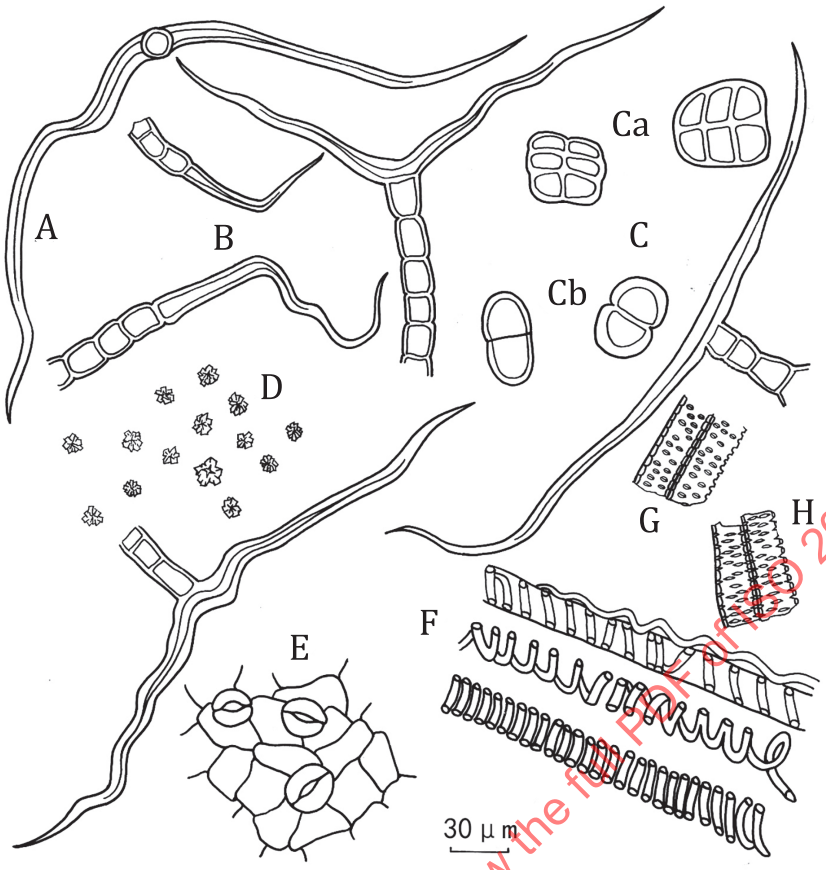

Key

A T-shaped hair
B uniseriate soft hair
C glandular hair
Ca glandular hair in lateral view
Cb glandular hair in apical view
D cluster crystal of calcium oxalate
E fragment of epidermis in surface view
F spiral vessel
G vessel with bordered pit
H reticulate vessel

Figure 2 — illustration of powdered *Artemisia argyi* leaf

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5.4 Thin layer chromatogram

The chromatogram of the sample solution shall exhibit a purplish-red spot corresponding to caryophyllene oxide at the same Rf value (0,55 ± 0,05) as caryophyllene oxide from the reference solution, and exhibit main spots with the same colours corresponding to those from the reference medicine solution at the same Rf values.

5.5 Volatile oil and 1,8-cineole ($C_{10}H_{18}O$) content

The volatile oil content and the mass fraction of 1,8-cineole should be detected.

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5.6 Total flavonoids content

The mass fraction of total flavonoids, expressed as apigenin ($C_{15}H_{10}O_5$), should be detected.

5.7 Dilute ethanol-soluble extract

The mass fraction of dilute ethanol-soluble extract shall not be less than 12,0 %.

5.8 Moisture content

The mass fraction of moisture shall not be more than 15,0 %.

5.9 Total ash

The mass fraction of total ash shall not be more than 12,0 %.

5.10 Acid-insoluble ash

The mass fraction of acid-insoluble ash shall not be more than 3,0 %.

6 Sampling

The maximum weight of each batch of the material should not be more than 5 000 kg. When a batch consists of two or more containers or packaging units, the container or packaging number of sampling should be based on the World Health Organization: *Quality control methods for herbal materials*, *General advice on sampling*. Take three original samples from the top, middle and bottom of each container or package selected. These original samples should be combined into a pooled sample and mixed carefully.

The average sample is obtained by quartering. Adequately mix the pooled sample into an even and square-shaped heap, and divide it diagonally into four equal parts. Take two diagonally opposite parts and mix carefully. Repeat the process as necessary until the required quantity (not less than 1 000 g) is obtained. Then, using the same quartering procedure, divide the average sample into four final samples, taking care that each portion is representative of the bulk material. Use final samples as the test sample (not less than 250 g) for the measurement and analysis. A portion of each final sample should be retained to serve as reference material, which may also be used for re-test purposes if necessary.

Use cut samples in the experiments except macroscopic identification and microscopic identification. Before the experiment, the test sample should be cut finely into pieces of about 4 mm^2, mixed carefully and sealed.

7 Test methods

7.1 Macroscopic identification

Not less than 200 g of the test sample is used to observe by the naked eyes. Observe the glandular dots and small pits on the upper surface of the leaf blade by using a stereomicroscope. Smell the odour of the sample, taste its flavor, and measure the length and width of the unfolded leaves after immersing them in water.

7.2 Microscopic identification

Take about 1 mg of the powdered test sample on a glass slide, add two to 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, and put a cover glass on it. Then, observe the microscopic characteristics using an optical microscope.

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7.3 Thin-layer chromatographic identification

The testing method described in Annex A applies.

7.4 Determinations of volatile oil content and 1,8-cineole($C_{10}H_{18}O$) content

The testing methods described in Annex B apply.

7.5 Determination of total flavonoids content

The testing method described in Annex C applies.

7.6 Determination of dilute ethanol-soluble extract

Use a volume fraction of 50 % ethanol solution as the dilute ethanol solvent. Take 2,00 g of the test sample, and extract with 70 ml of dilute ethanol in a suitable flask with occasional shaking for 5 h. Allow to stand for 16 h, filter, and 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, dry at 105 °C for 4 h, and cool in a desiccator (silica gel). Weigh it accurately, multiply the weight 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 Determination of moisture content

Take 20,00 g of the test sample and determine the moisture content in accordance with the method specified in ISO 939.

7.8 Determination of total ash

The testing method specified in ISO 928 applies.

7.9 Determination of acid-insoluble ash

The testing method specified in ISO 930 applies.

8 Test report

For each test sample, the test report shall record the sample quantity, sampling date, supplier's name, as well as the producing area and commodity name in detail.

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

a) the sampling method used;

b) the test methods used, with reference to Clause 7 of this document;

c) all operating details not specified in this document, together with details of any incidents which may have influenced the test result(s), e.g. temperature, humidity, instrument model, etc.;

d) anything unusual observed in the process of the test;

e) the date of the test;

f) the test result(s).

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9 Packaging, storage and transportation

The packaging shall not transmit any odour or flavor to the product and shall not contain substances which may damage the product or form a health risk.

The herbal medicine, *Artemisia argyi* leaf, shall be sealed and stored in a dry, shady and cool place. The storage temperature shall not be over 25 °C, and the relative humidity shall not be over 65 %.

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

10 Marking and labelling

The following items and information shall be marked or labelled on the package:

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

b) all the quality features indicated in 5.5 to 5.8 and determined in accordance with the methods specified in Clause 7;

c) the net weight or minimum weight of the herbal medicine contained in the package;

d) the producing area of the herbal medicine, province/state and country, as well as the name or trademark or logo of the producer and supplier;

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

f) storage and transit requirements;

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

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Annex A
(normative)

Thin layer chromatographic identification of *Artemisia argyi* leaf

A.1 Preparation of test solution

Weigh 2,0 g of the cut test sample into a flask, add 25 ml of petroleum ether (90 °C to 120 °C) and heat in a water bath under a reflux condenser for 30 min. Cool, filter, and evaporate the filtrate to dryness. Dissolve the residue with 1 ml of n-hexane, and use supernatant liquid as the sample solution.

A.2 Preparations of reference solution and reference medicine solution

Dissolve 2,5 mg of reference substance caryophyllene oxide in 5 ml of n-hexane to obtain the reference solution. Take 1,0 g of reference medicine of *Artemisia argyi* leaf, followed with the operation in A.1 to obtain the reference medicine solution.

A.3 Preparation of the chromogenic agent

Slowly add 25 ml with a volume fraction of 20 % sulfuric acid solution to 25 ml of cold glacial acetic acid, then mix with 2,5 ml of 4-methoxybenzaldehyde and 50 ml with a volume fraction of 20 % sulfuric acid solution to obtain the chromogenic agent.

A.4 Identification

Spot respectively 5 µl of the three solutions above on a same silica gel plate which is previously dried in an oven at 105 °C for 30 min. Develop the plate with a mixture of petroleum ether (60 °C to 90 °C), methylbenzene and acetone (10:8:0,5) over a path of 15 cm. Take the plate out and dry in air. Spray evenly with the chromogenic agent, and heat at 105 °C until the spot colour looks clear. Identify the sample by comparing the number, Rf values and colours of the spots in chromatogram obtained from the test solution with those from the reference solution and reference medicine solution in daylight. A typical reference TLC chromatogram is shown in Figure A.1, in which caryophyllene oxide from each of the three solutions above shows a purplish-red spot with an Rf value of 0,55 ± 0,05.

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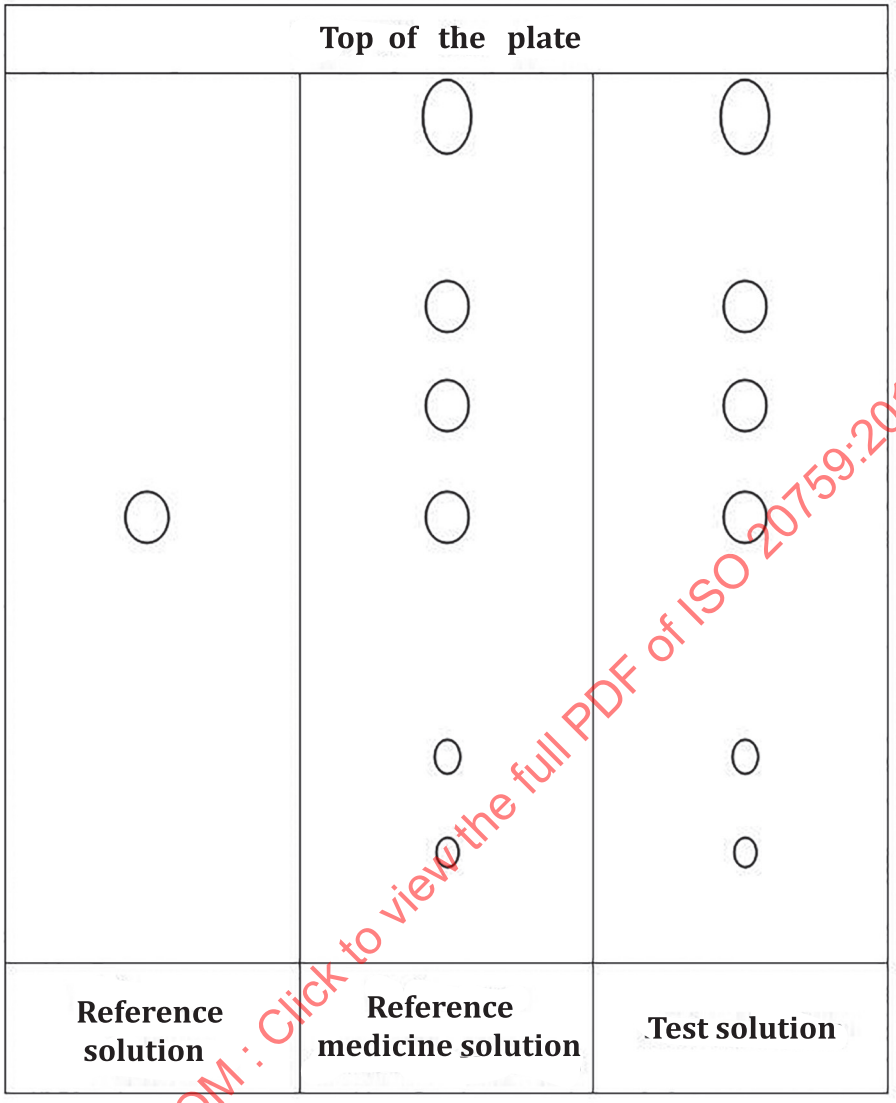

Figure A.1 — Schematic diagram of typical TLC chromatogram of *Artemisia argyi* leaf

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Annex B
(normative)

Determination of volatile oil and 1,8–cineole contents in *Artemisia argyi* leaf

B.1 Determination of volatile oil content

Take 40,00 g of the cut test sample, and determine the volatile oil content in accordance with the method specified in ISO 6571. In determination, the volume of water added into the flask is 600 ml, and the distillation time is 5 h. The volatile oil content should not be less than 0,30 ml/100g, which should be calculated on the dried basis.

B.2 Determination of 1,8–cineole content

B.2.1 Preparation of reference solution

Dissolve a proper amount of reference substance 1,8-cineole in n-hexane to obtain the reference solution containing 0,15 mg of 1,8-cineole in each millilitre.

B.2.2 Preparation of test solution

Weigh 2,500 g of the cut test sample into a conical flask with a glass-stopper. Add 25,0 ml of n-hexane, accurately weigh, heat under a reflux condenser for 1 h, then cool and weigh again. Replenish the loss of solvent with n-hexane, shake and filter. Take the subsequent filtrate as the test solution.

B.2.3 Chromatographic system

Detector	Flame ionization
Column	Material is fused silica, with a length of 30 m, a film thickness of 0,25 µm and an internal diameter of 0,25 mm.
Stationary phase	Macrogol with an average molecular mass of 15 000
Carrier gas	Nitrogen for chromatography
Flow rate	1,0 ml/min
Split ratio	1:10
Injection size	1 µl
Column temperatures	90 °C from 0 min to 8 min; 90 °C to 180 °C from 8 min to 11 min, with a rate of 30 °C/min; 180 °C from 11 min to 16 min
Injection port temperature	230 °C
Detector temperature	250 °C
System suitability	Number of theoretical plates should not be less than 10 000, calculated for the peak due to 1,8-cineole in the chromatogram.

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B.2.4 Detection and content calculation

Draw accurately 1 µl of the reference solution and 1 µl of the test solution, and perform the test under the above conditions. Determine the peak area of 1,8-cineole by the automatic integration method. Calculate the mass fraction of 1,8-cineole which should not be less than 0,040 % (on the dried basis) using Formula (B.1):

$$C_x = C_r \times A_x/A_r \quad \text{(B.1)}$$

where

C_x is the concentration of 1,8-cineole in the test solution;

C_r is the concentration of 1,8-cineole in the reference solution;

A_x is the area of the peak due to 1,8-cineole in the chromatogram obtained with the test solution;

A_r is the area of the peak due to 1,8-cineole in the chromatogram obtained with the reference solution.

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