Artemisiae Argyi Folium

Figure 1 A photograph of Artemisiae Argyi Folium

A. Artemisiae Argyi Folium B. Upper surface of laminae
C. Lower surface of laminae

1. NAMES

Official Name: Artemisiae Argyi Folium

Chinese Name: 艾葉

Chinese Phonetic Name: Aiye

2. SOURCE

Artemisiae Argyi Folium is the dried leaf of *Artemisia argyi* Lévl. et Vant. (Asteraceae). The leaf is collected in summer before flowering stage, foreign matter removed, then dried under the sun to obtain Artemisiae Argyi Folium.

3. DESCRIPTION

Mostly crumpled and broken, with short petiole. Lamina ovate-elliptical when intact and flattened, pinnatipartite, segments elliptical-lanceolate, margin dentate irregular; upper surface greyish-green to dark yellowish-green, sparsely pubescent and glandular-punctate; lower surface densely covered with greyish-white tomentose. Texture soft. Odour delicately aromatic; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, covered with cuticles. Non-glandular hairs and glandular hairs present abundantly on the upper and lower surface of epidermis, non-glandular hairs especially abundant on the lower epidermis. Non-glandular hairs 2 types: T-shaped and uniseriate, both frequently broken. Collenchyma consists of 2-3 layers of cells, underneath the epidermis of the midrib. Palisade tissue and spongy tissue each comprises half of the lamina. Palisade tissue consists of 1 layer of cells, loosely packed. Midvein 1 distinctly protruded, collateral, with sclerenchymatous cells on the upper and lower parts of the vascular bundle. Clusters of calcium oxalate scattered in mesophyll cells, rosette-aggregated (Fig. 2).

Powder

Colour greenish-brown. Non-glandular hairs 2 types, numerous, first type T-shaped, with elongated and bent apical cell and 2- to 5-celled stalk, unequal arms 2, 91-422 μm long; second type uniseriate, 3- to 5-celled, with very long and twisted apical cell, frequently broken. Glandular hairs consist of 4-6 oppositely overlapped cells, without stalk, parameciu-like in surface view, 32-53 μm long and 19-31 μm wide. Stomata anomocytic, 21-41 μm long, 19-30 μm wide. Oil droplets present in palisade and mesophyll tissues. Clusters of calcium oxalate 3-12 μm in diameter, rosette-aggregated; polychromatic under the polarized microscope (Fig. 3).

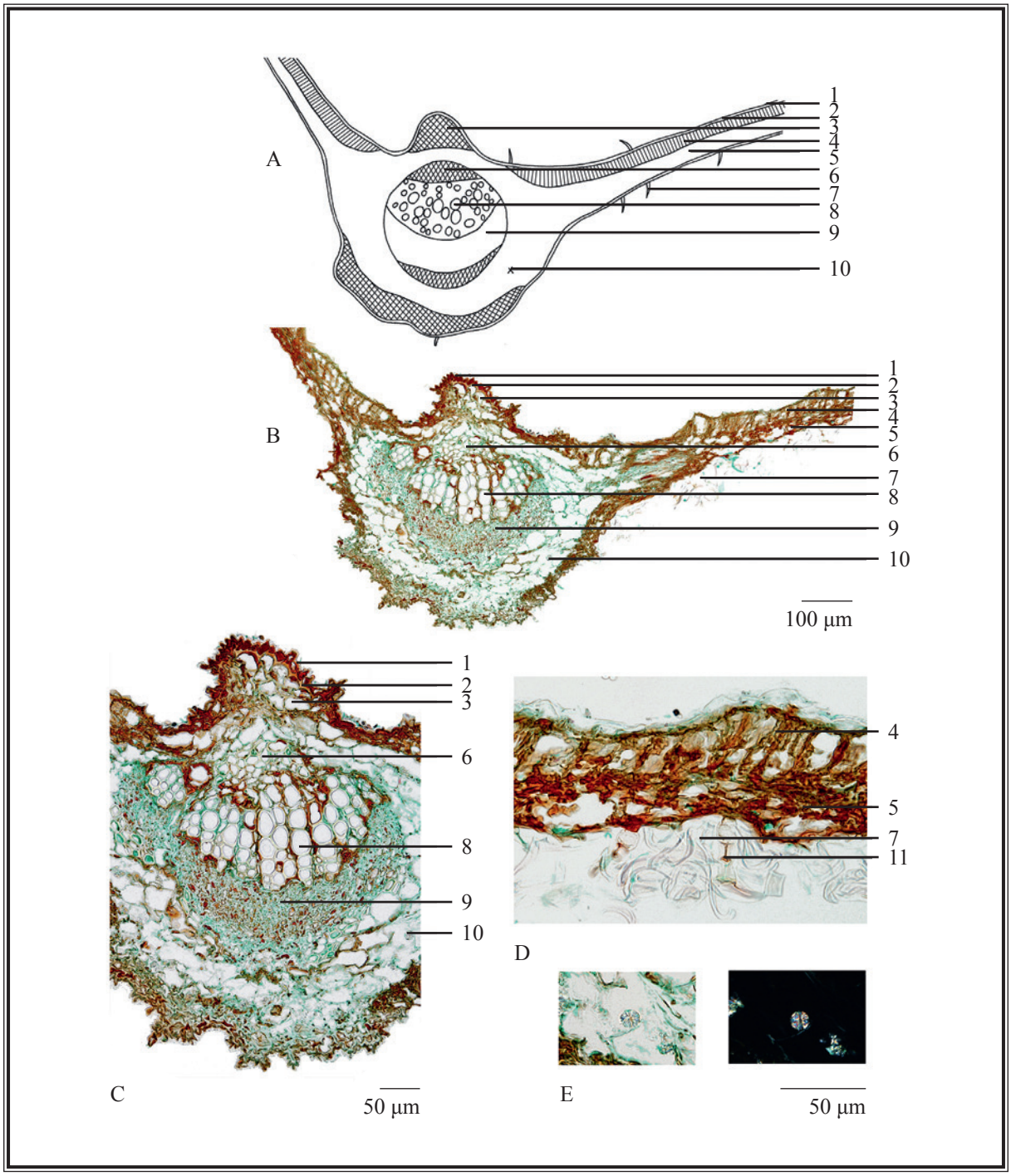


Figure 2 Microscopic features of transverse section of Artemisiae Argyi Folium

A. Sketch B. Section illustration C, D. Section magnified E. Clusters of calcium oxalate

1. Cuticles 2. Epidermis 3. Collenchyma 4. Palisade tissue
5. Spongy tissue 6. Sclerenchyma 7. Uniseriate non-glandular hair
8. Xylem 9. Phloem 10. Clusters of calcium oxalate 11. T-shaped non-glandular hair

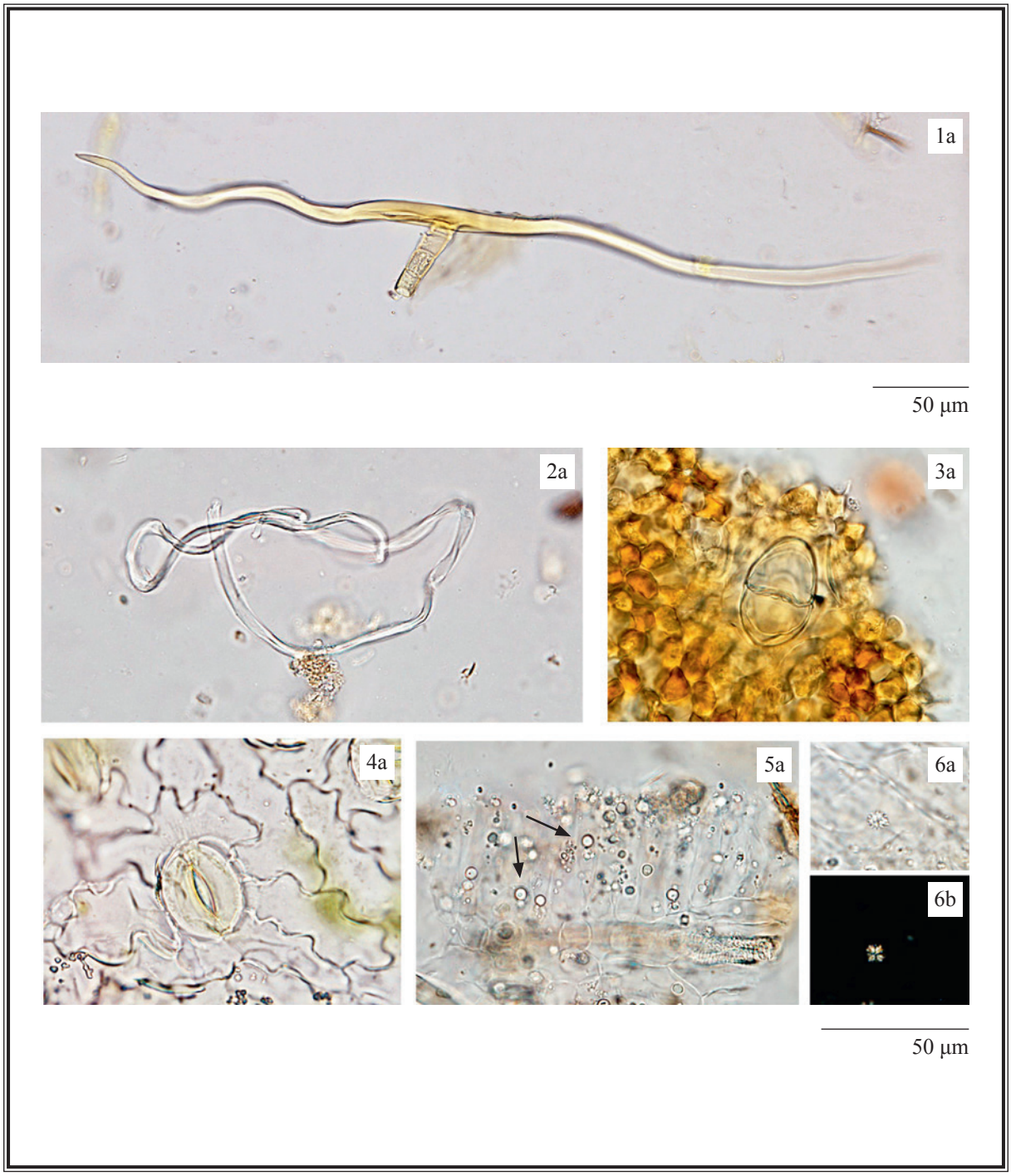


Figure 3 Microscopic features of powder of Artemisiae Argyi Folium

1. T-shaped non-glandular hair 2. Uniseriate non-glandular hair 3. Glandular hair
4. Stomata 5. Oil droplets (occurring in palisade and mesophyll tissues)
6. Cluster of calcium oxalate

a. Features under the light microscope b. Features under the polarized microscope

4.2 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solution

Caryophyllene oxide standard solution

Weigh 1.5 mg of caryophyllene oxide CRS (Fig. 4) and dissolve in 10 mL of n-hexane.

Developing solvent system

Prepare a mixture of n-hexane and acetone (20:1, v/v).

Spray reagent

Mix cautiously 25 mL of sulphuric acid (20%, v/v) into 25 mL of ice-cold glacial acetic acid. Add 2.5 mL of *p*-anisaldehyde. Add further 50 mL of sulphuric acid (20%, v/v).

Test solution

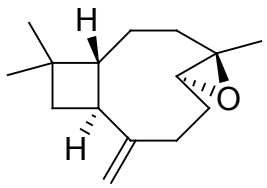
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of n-hexane. Sonicate (220 W) the mixture for 30 min. Filter and add 0.5 g of activated charcoal to the filtrate. Allow to stand for about 15 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of n-hexane.

Procedure

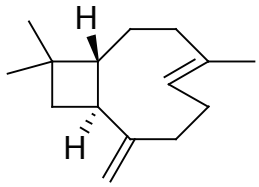
Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately caryophyllene oxide standard solution (5 µL) and the test solution (4 µL) to the plate. Develop over a path of about 8 cm. After the development, remove 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 15 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of caryophyllene oxide.

(i)



(ii)



(iii)

Figure 4 Chemical structures of (i) caryophyllene oxide (ii) *trans*-caryophyllene and (iii) 1,8-cineole

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Caryophyllene oxide standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of caryophyllene oxide CRS and dissolve in 10 mL of n-hexane.

Trans-caryophyllene standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of *trans*-caryophyllene CRS (Fig. 4) and dissolve in 10 mL of n-hexane.

1,8-Cineole standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 0.3 mg of 1,8-cineole CRS (Fig. 4) and dissolve in 10 mL of n-hexane.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 25-mL conical flask, then add 10 mL of n-hexane. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 10-mL volumetric flask. Make up to the mark with n-hexane. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-5, 0.32 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 µm thick. The injection temperature is at 250°C. The detector temperature is at 250°C. The split injection mode at a ratio of 5:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 30	60 → 210	5

System suitability requirements

Perform at least five replicate injections, each using 2 µL of caryophyllene oxide Std-FP, *trans*-caryophyllene Std-FP and 1,8-cineole Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole should not be more than 5.0%; the RSD of the retention times of caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole peaks should not be more than 2.0%; the column efficiencies determined from caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole peaks should not be less than 1000000, 700000 and 200000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 3 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject caryophyllene oxide Std-FP, *trans*-caryophyllene Std-FP, 1,8-cineole Std-FP and the test solution (2 µL each) into the GC system and record the chromatograms. Measure the retention times of caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole peaks in the chromatograms of caryophyllene oxide Std-FP, *trans*-caryophyllene Std-FP, 1,8-cineole Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of caryophyllene oxide Std-FP, *trans*-caryophyllene Std-FP and 1,8-cineole Std-FP. The retention times of caryophyllene oxide, *trans*-caryophyllene and 1,8-cineole peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of Artemisiae Argyi Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Artemisiae Argyi Folium extract

Peak No.	RRT	Acceptable Range
1 (1,8-cineole)	0.47	± 0.03
2	0.66	± 0.03
3 (marker, *trans*-caryophyllene)	1.00	-
4 (caryophyllene oxide)	1.20	± 0.03
5	1.28	± 0.03

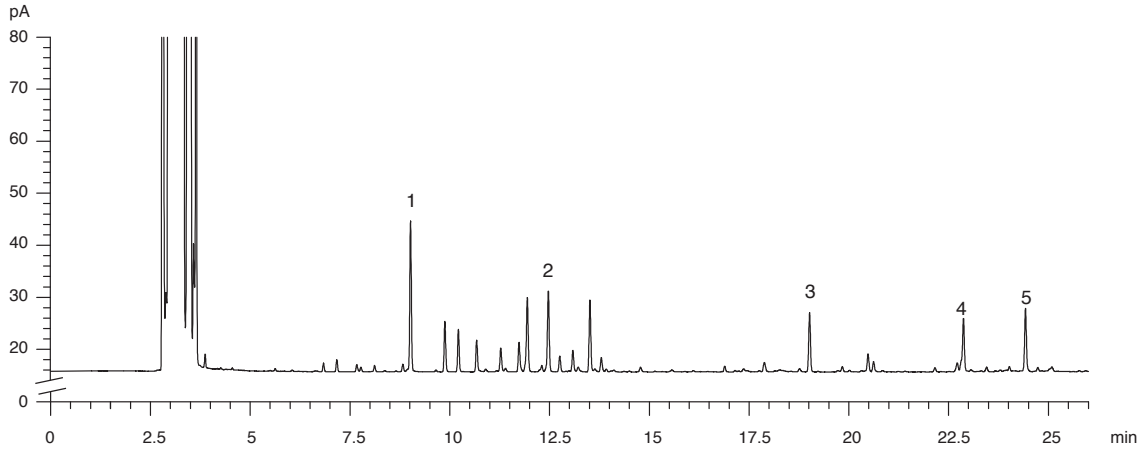


Figure 5 A reference GC fingerprint chromatogram of Artemisiae Argyi Folium extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference GC fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.0%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(C).

Standard solution

Mixed caryophyllene oxide and trans-caryophyllene standard stock solution, Std-Stock (20 mg/L each)
Weigh accurately 2.0 mg of caryophyllene oxide CRS and 2.0 mg of *trans*-caryophyllene CRS, and dissolve in 100 mL of n-hexane.
Mixed caryophyllene oxide and trans-caryophyllene standard solution for assay, Std-AS
Measure accurately the volume of the mixed caryophyllene oxide and *trans*-caryophyllene Std-Stock, dilute with n-hexane to produce a series of solutions of 0.1, 1, 2, 3 and 5 mg/L for both caryophyllene oxide and *trans*-caryophyllene.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 15 mL of n-hexane. Cap the conical flask. Sonicate (220 W) the mixture for 30 min in a water bath at about 15°C. Allow to stand at room temperature for 15 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with 1 mL of n-hexane. Combine the solutions and cap the volumetric flask. Repeat the extraction for two more times. Combine the solutions and make up to the mark with n-hexane. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (HP-5, 0.32 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 µm thick. The injection temperature is at 250°C. The detector temperature is at 250°C. The splitless injection mode is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (ºC)	Rate (ºC/min)
0 – 30	60 → 210	5

System suitability requirements

Perform at least five replicate injections, each using 3 µL of the mixed caryophyllene oxide and *trans*-caryophyllene Std-AS (2 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of caryophyllene oxide and *trans*-caryophyllene should not be more than 5.0%; the RSD of the retention times of caryophyllene oxide and *trans*-caryophyllene peaks should not be more than 2.0%; the column efficiencies determined from caryophyllene oxide and *trans*-caryophyllene peaks should not be less than 400000 and 300000 theoretical plates respectively.

The *R* value between caryophyllene oxide peak and the closest peak; and the *R* value between *trans*-caryophyllene peak and the closest peak in the chromatogram of the test solution should not be less than 1.3 and 1.5 respectively.

Calibration curves

Inject a series of the mixed caryophyllene oxide and *trans*-caryophyllene Std-AS (3 µL each) into the GC system and record the chromatograms. Plot the peak areas of caryophyllene oxide and *trans*-caryophyllene against the corresponding concentrations of the mixed caryophyllene oxide and *trans*-caryophyllene Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 3 µL of the test solution into the GC system and record the chromatogram. Identify caryophyllene oxide and *trans*-caryophyllene peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed caryophyllene oxide and *trans*-caryophyllene Std-AS. The retention times of caryophyllene oxide and *trans*-caryophyllene peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of caryophyllene oxide

and *trans*-caryophyllene in the test solution, and calculate the percentage contents of caryophyllene oxide and *trans*-caryophyllene in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.036% of the total content of caryophyllene oxide ($C_{15}H_{24}O$) and *trans*-caryophyllene ($C_{15}H_{24}$), calculated with reference to the dried substance.