Myristicae Semen

Figure 1 A photograph of Myristicae Semen

A. Myristicae Semen

B. Magnified image of shrunken embryo (→)

C. Magnified image of transverse section of kernel

1. NAMES

Official Name: Myristicae Semen

Chinese Name: 肉豆蔻

Chinese Phonetic Name: Roudoukou

2. SOURCE

Myristicae Semen is the dried kernel of *Myristica fragrans* Houtt. (Myristicaceae). The kernel is collected in summer and winter, foreign matter removed, washed clean, then dried at around 45ºC to obtain Myristicae Semen.

3. DESCRIPTION

Ovoid-globose to ellipsoid, one end smoothly rounded, the other end vaguely truncate, 1.7-3.4 cm long, 13-23 mm in diameter. Externally greyish-brown to greyish-yellow, sometimes covered with white powder (lime powder). Kernel covered with pale longitudinal furrows and irregular reticulated wrinkles. A protuberant hilum is located at the truncated end, yellowish-white; chalaza dark and dented. Raphe longitudinally furrowed, connecting the two ends. Texture hard, fracture showing marble-like striations, brownish-yellow, interweaved. A dried and shrunken embryo visible at the truncated end, oily. Odour strongly fragrant; taste pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer layers of perisperm consist of about 10 layers of tangentially flattened and wrinkled cells, containing brown contents (before stained), scattered with small vascular bundles; the dark brown perisperm (before stained) penetrates into endosperm, forming marble-like striations; oil cells numerous, 25-125 μm in diameter. Endosperm cells subrounded, walls thin, filled with starch granules, oil droplets and aleurone grains, scattered with pale yellow cells (before stained) (Fig. 2).

Powder

Colour reddish-brown to brown. Fatty oil abundant, becoming oil droplets when heated, turning into raphide-shaped cluster or feather-shaped crystals when cooled. Perisperm cells in pieces, polygonal, containing brownish-red, bright red or yellowish-brown pigments. Endosperm cells yellow, subpolygonal, filled with starch granules, oil droplets and aleurone grains. Starch granules mainly simple starch granules, subrounded, 10-25 μm in diameter, hilum dotted, slit-shaped or asteroidal; black and cruciate-shaped under the polarized microscope; compound starch granules few, composed of 2-8 units, 15-35 μm in diameter, hilum distinct. Vessels mainly spiral, reticulate vessels also found, yellow, 10-25 μm in diameter (Fig. 3).

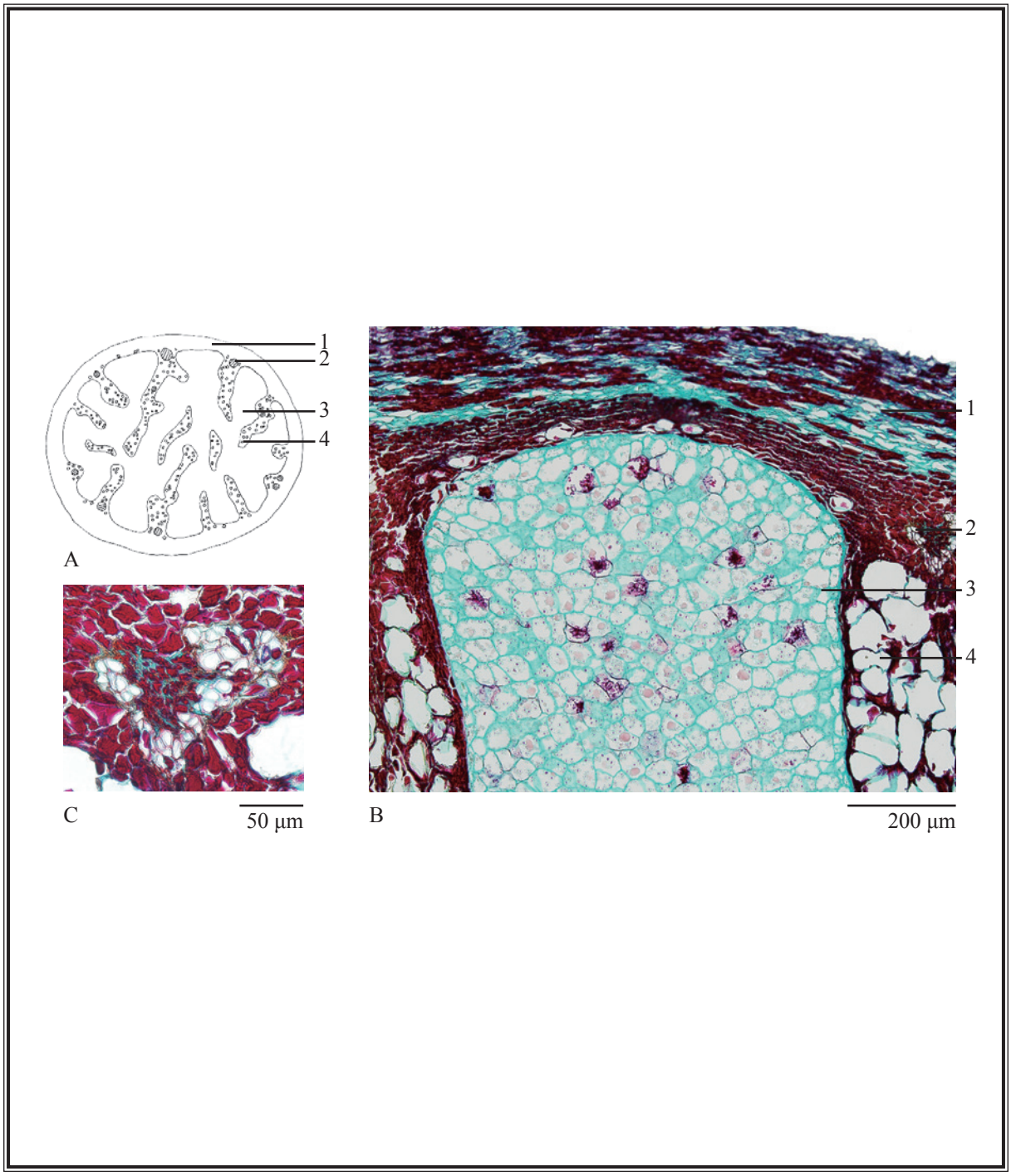

Figure 2 Microscopic features of transverse section of Myristicae Semen

A. Sketch B. Section illustration C. Vascular bundle

1. Perisperm 2. Vascular bundle 3. Endosperm 4. Oil cell

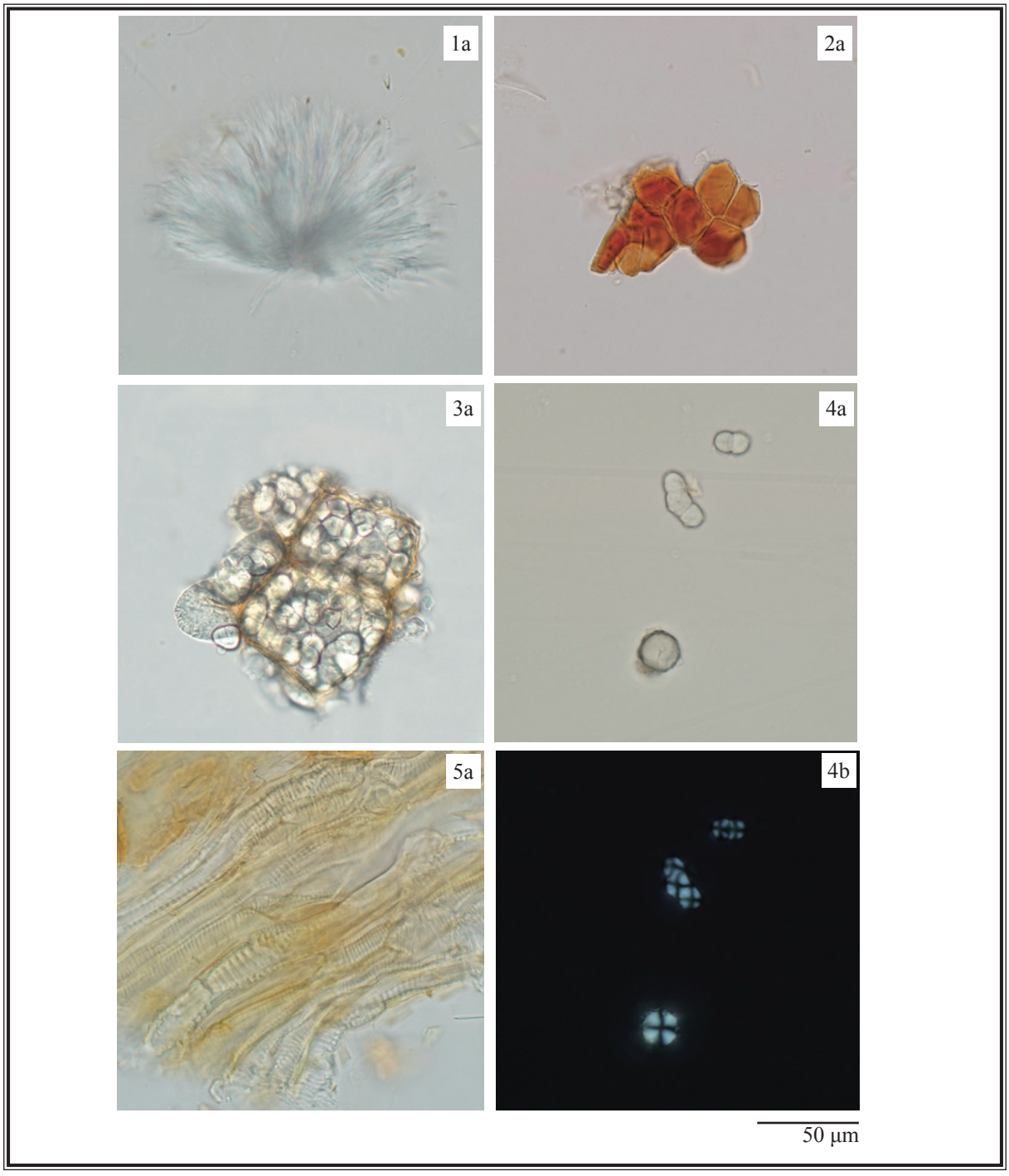

Figure 3 Microscopic features of powder of Myristicae Semen

1. Fatty oil 2. Perisperm cells 3. Endosperm cells 4. Starch granules 5. Vessels

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

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

Standard solution

Myristicin standard solution

Weigh 1.0 mg of myristicin CRS (Fig. 4) and dissolve in 1 mL of methanol. Keep at about 4ºC.

Developing solvent system

Prepare a mixture of cyclohexane and ethyl acetate (19:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve in 5 g of vanillin.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 25 mL of petroleum ether (60-80ºC). Sonicate (250 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately myristicin standard solution (2 μL) and the test solution (4 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 7 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 105ºC until the spots or bands become visible (about 5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

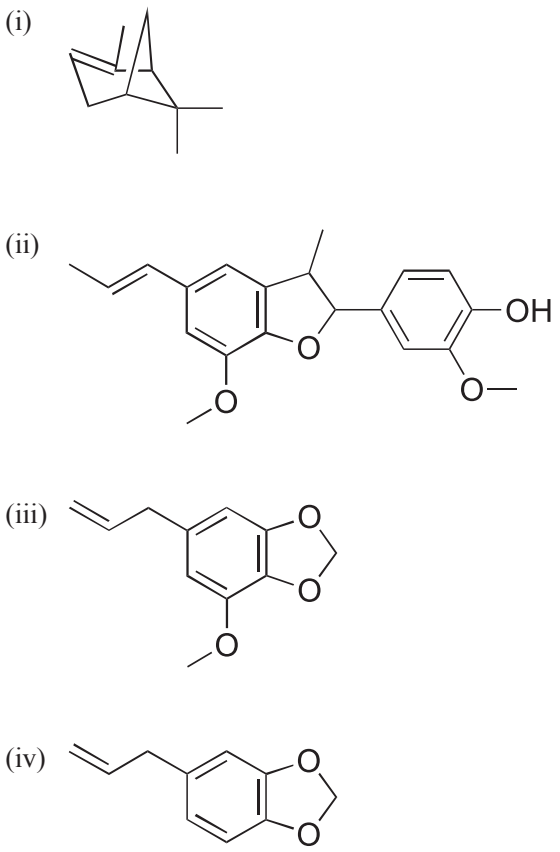

Figure 4 Chemical structures of (i) α-pinene (ii) dehydrodiisoeugenol (iii) myristicin and (iv) safrole

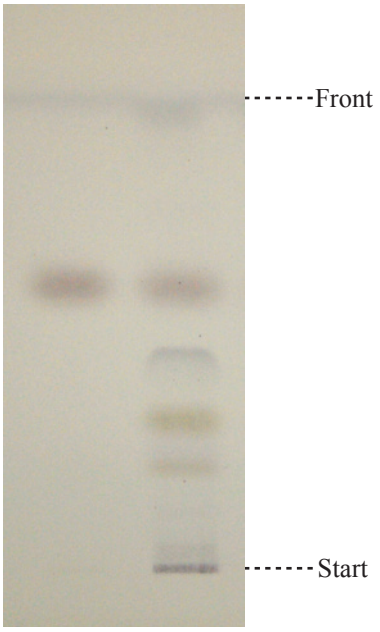

Figure 5 A reference HPTLC chromatogram of Myristicae Semen extract observed under visible light after staining

1. Myristicin standard solution 2. Test solution

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 myristicin (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Myristicin standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of myristicin CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

α-Pinene standard solution for fingerprinting, Std-FP (130 mg/L)

Weigh 1.3 mg of α-pinene CRS (Fig. 4) and dissolve in 10 mL of methanol. Keep at about 4ºC.

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

Weigh 0.3 mg of safrole CRS (Fig. 4) and dissolve in 10 mL of methanol. Keep at about 4ºC.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 20 mL of *n*-hexane. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with *n*-hexane. Combine the solutions and make up to the mark with *n*-hexane. Filter through a 0.45-μm nylon 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 220ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 1	40	-
1 – 37	40 → 220	5
37 – 47	220	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of myristicin Std-FP, α-pinene Std-FP and safrole Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of myristicin, α-pinene and safrole should not be more than 5.0%; the RSD of the retention times of myristicin, α-pinene and safrole peaks should not be more than 2.0%; the column efficiencies determined from myristicin, α-pinene and safrole peaks should not be less than 250000, 25000 and 100000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; the *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject myristicin Std-FP, α-pinene Std-FP, safrole Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention times of myristicin, α-pinene and safrole peaks in the chromatograms of myristicin Std-FP, α-pinene Std-FP, safrole Std-FP and

the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify myristicin, α-pinene and safrole peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of myristicin Std-FP, α-pinene Std-FP and safrole Std-FP. The retention times of myristicin, α-pinene and safrole 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 Myristicae Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Myristicae Semen extract

Peak No.	RRT	Acceptable Range
1 (α-pinene)	0.28	± 0.03
2 (γ-terpinene)	0.43	± 0.03
3 (terpinen-4-ol)	0.59	± 0.03
4 (safrole)	0.73	± 0.03
5 (marker, myristicin)	1.00	-

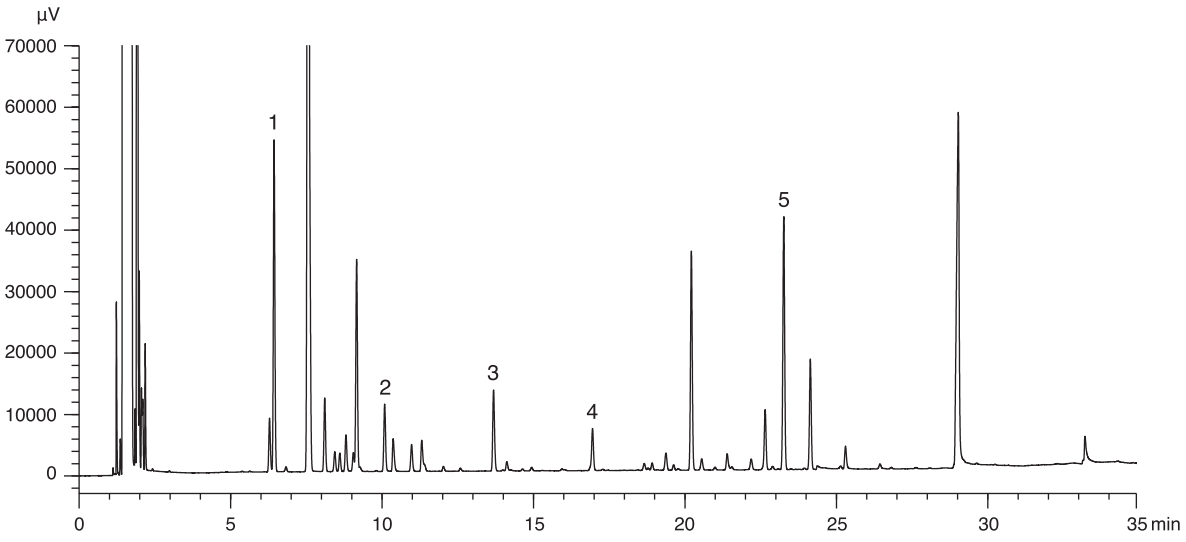

Figure 6 A reference GC fingerprint chromatogram of Myristicae Semen extract

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. 6).

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 2.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 11.0%.

Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

7.1 Assay of Dehydrodiisoeugenol

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

Standard solution

Dehydrodiisoeugenol standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of dehydrodiisoeugenol CRS (Fig. 4) and dissolve in 10 mL of methanol. Keep at about 4ºC.

Dehydrodiisoeugenol standard solution for assay, Std-AS

Measure accurately the volume of the dehydrodiisoeugenol Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 30, 60, 100 mg/L for dehydrodiisoeugenol. Keep at about 4ºC.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 100-mL conical flask, then add 20 mL of methanol. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with methanol. Combine the solutions and make up to the mark with methanol. Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (274 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (70:30, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of dehydrodiisoeugenol Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of dehydrodiisoeugenol should not be more than 5.0%; the RSD of the retention time of dehydrodiisoeugenol peak should not be more than 2.0%; the column efficiency determined from dehydrodiisoeugenol peak should not be less than 5000 theoretical plates.

The *R* value between dehydrodiisoeugenol peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of dehydrodiisoeugenol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of dehydrodiisoeugenol against the corresponding concentrations of dehydrodiisoeugenol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify dehydrodiisoeugenol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of dehydrodiisoeugenol Std-AS. The retention times of dehydrodiisoeugenol peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of dehydrodiisoeugenol in the test solution, and calculate the percentage content of dehydrodiisoeugenol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.13% of dehydrodiisoeugenol ($C_{20}H_{22}O_4$), calculated with reference to the dried substance.

7.2 Assay of Myristicin and Safrole

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

Standard solution

Mixed myristicin and safrole standard stock solution, Std-Stock (1400 mg/L for myristicin and 600 mg/L for safrole)

Weigh accurately 14.0 mg of myristicin CRS and 6.0 mg of safrole CRS, and dissolve in 10 mL of methanol. Keep at about 4ºC.

Mixed myristicin and safrole standard solution for assay, Std-AS

Measure accurately the volume of the mixed myristicin and safrole Std-Stock, dilute with methanol to produce a series of solutions of 70, 140, 210, 350, 700 mg/L for myristicin and 6, 12, 30, 60, 120 mg/L for safrole. Keep at about 4ºC.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 20 mL of *n*-hexane. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with *n*-hexane. Combine the solutions and make up to the mark with *n*-hexane. Filter through a 0.45-µm nylon 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 220ºC. The detector temperature is at 250ºC. The split injection mode at a ratio of 10:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 1	40	-
1 – 37	40 → 220	5
37 – 47	220	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of the mixed myristicin and safrole Std-AS (210 mg/L for myristicin and 30 mg/L for safrole). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of myristicin and safrole should not be more than 5.0%; the RSD of the retention times of myristicin and safrole peaks should not be more than 2.0%; the column efficiencies determined from myristicin and safrole peaks should not be less than 250000 and 100000 theoretical plates respectively.

The *R* value between myristicin peak and the closest peak; and the *R* value between safrole peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed myristicin and safrole Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of myristicin and safrole against the corresponding concentrations of the mixed myristicin and safrole Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify myristicin and safrole peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed myristicin and safrole Std-AS. The retention times of myristicin and safrole peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of myristicin and safrole in the test solution, and calculate the percentage contents of myristicin and safrole in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 1.1% of the total content of myristicin ($C_{11}H_{12}O_3$) and safrole ($C_{10}H_{10}O_2$), calculated with reference to the dried substance.

7.3 Assay of Volatile Oil

Weigh accurately 20 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 6.0% (v/w) of volatile oil.