Litseae Fructus

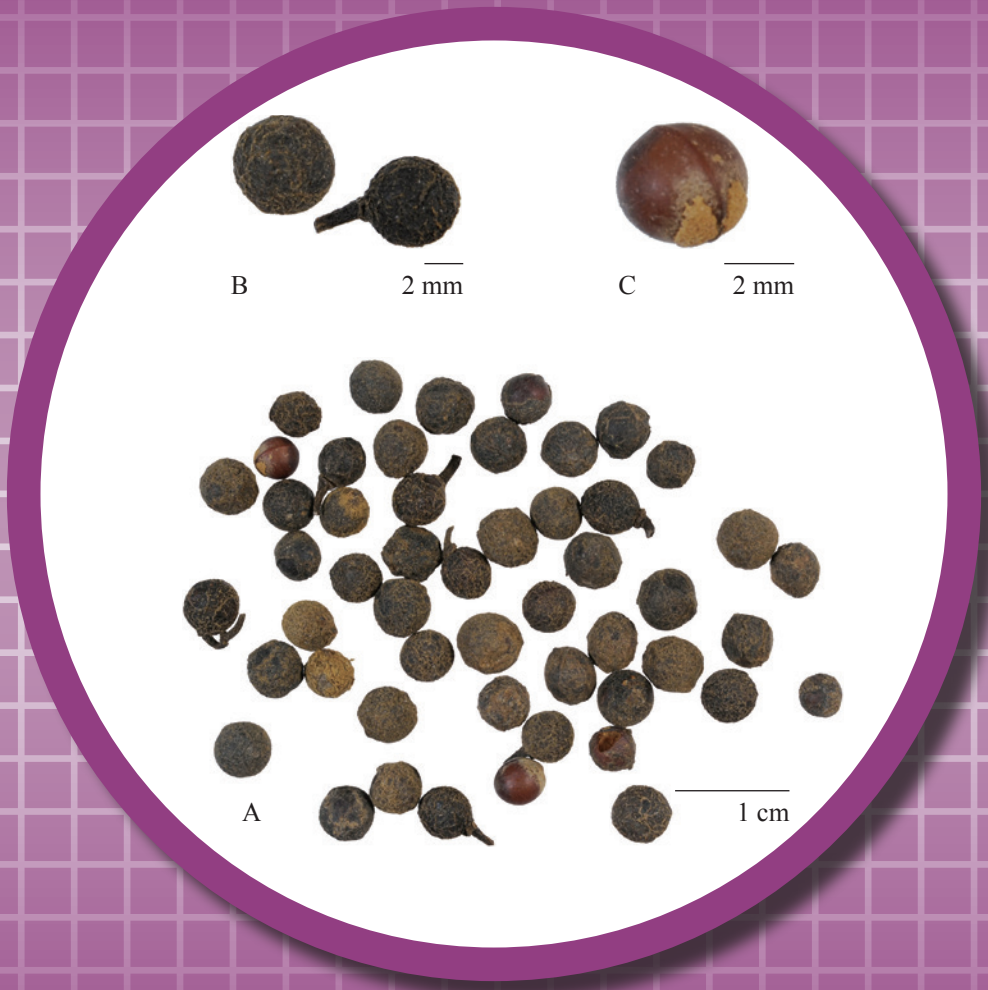

Figure 1 A photograph of Litseae Fructus

A. Litseae Fructus B. Magnified image of ripe fruit
C. Magnified image of seed with endocarp

1. NAMES

Official Name: Litseae Fructus

Chinese Name: 華澄茄

Chinese Phonetic Name: Bichengqie

2. SOURCE

Litseae Fructus is the dried ripe fruit of *Litsea cubeba* (Lour.) Pers. (Lauraceae). The fruit is collected in autumn when the fruit is ripe, foreign matter removed, then dried under the sun to obtain Litseae Fructus.

3. DESCRIPTION

Subspherical, 3-7 mm in diameter; externally brown to blackish-brown, with reticulated wrinkles. Occasionally the base bears a fine fruit stalk. A hard fragile kernel visible when the pericarp removed. Seed 1, cotyledons 2, yellowish-brown, oily. Odour fragrant; taste slightly pungent and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of cells, covered with thick cuticle. Mesocarp broad, cells subelliptical. Numerous oil cells mainly scattered on the outer side of the mesocarp; stone cells scattered singly or in groups on the inner side of the mesocarp. Endocarp narrow, consists of 4-6 layers of fusiform stone cells, palisade-like; prisms of calcium oxalate scattered in endocarp. Testa consists of several layers of parenchymatous cells. Degenerated endosperm consists of 1 layer of cells. Cotyledon 2, occupying the major portion of the transverse section, composed of subrounded cells (Fig. 2).

Powder

Colour brown. Stone cells of mesocarp scattered singly or in groups, rectangular to subrounded, 10-112 μm in diameter, walls thickened, lumens small, pits and pit canals distinct. Oil cells numerous, elliptic or rounded, 20-122 μm in diameter, containing yellowish-brown oil droplets. Stone cells of endocarp yellow, polygonal in top view, fusiform or arranged in palisade-like pattern in lateral view, lumens narrow and small. Prisms of calcium oxalate numerous, minute, 1-15 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

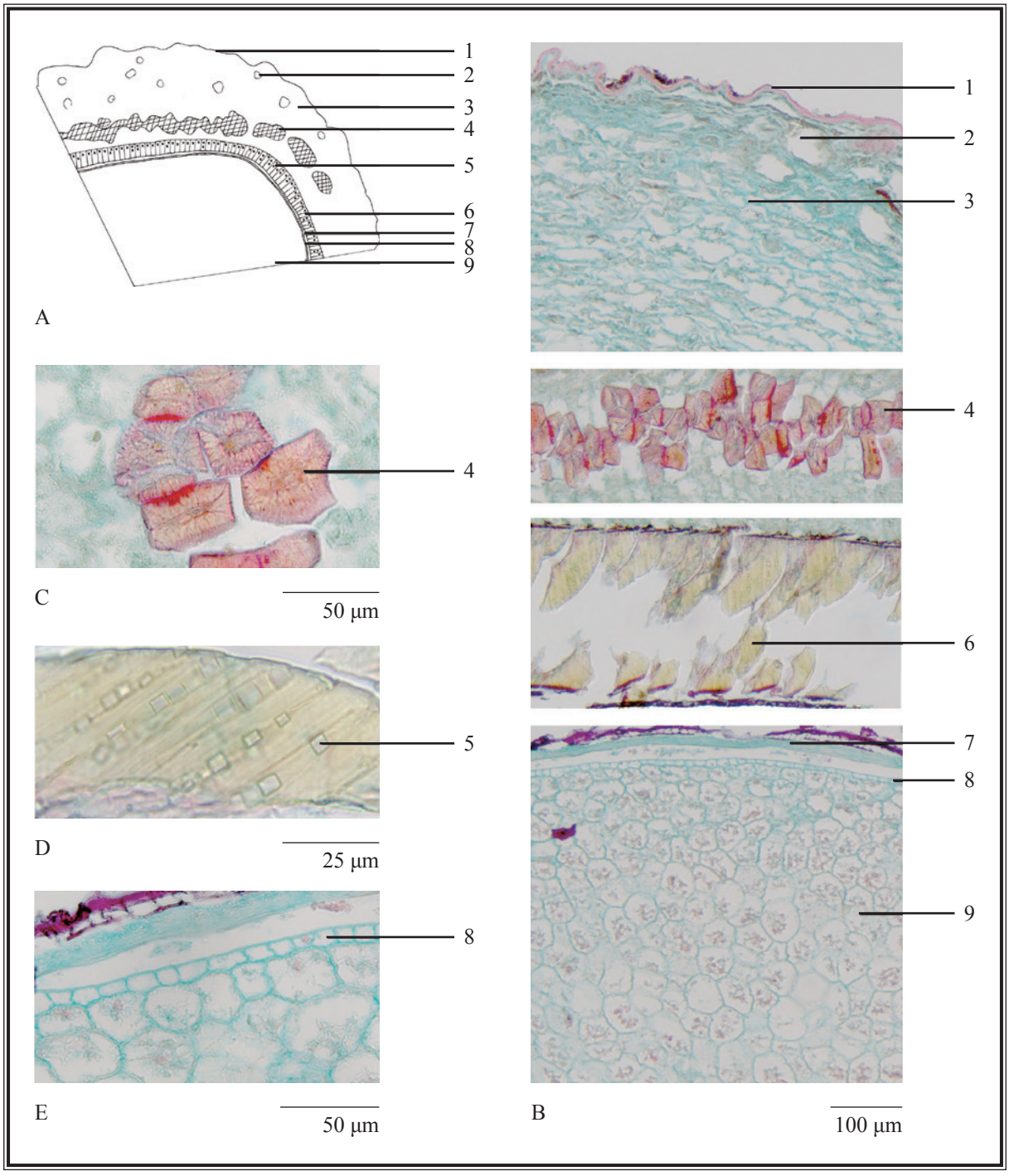

Figure 2 Microscopic features of transverse section of Litseae Fructus

A. Sketch B. Section illustration C. Stone cells of mesocarp D. Stone cells of endocarp E. Endosperm

1. Exocarp 2. Oil cells 3. Mesocarp 4. Stone cells of mesocarp 5. Prism of calcium oxalate 6. Endocarp 7. Testa 8. Endosperm 9. Cotyledon

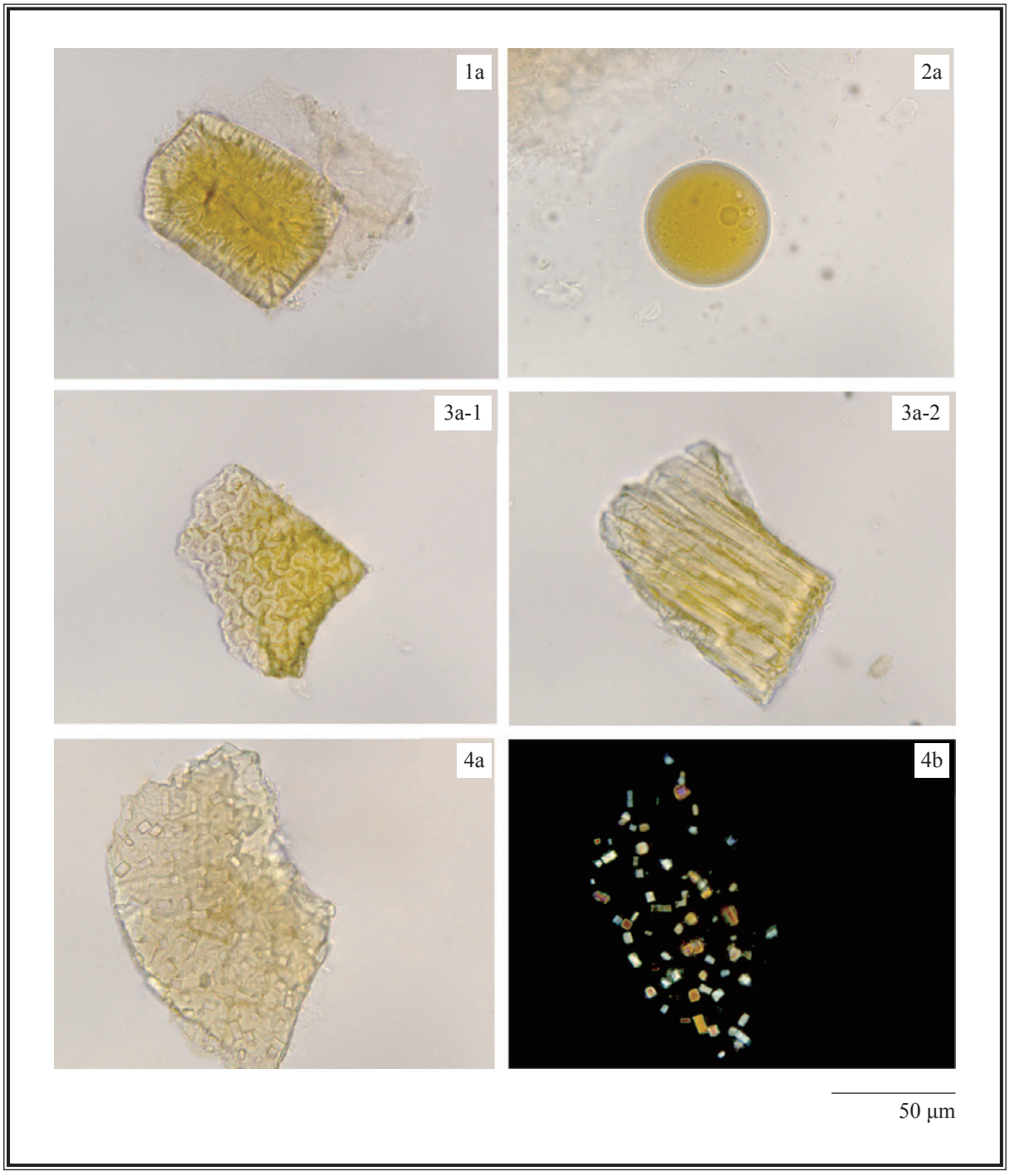

Figure 3 Microscopic features of powder of Litseae Fructus

1. Stone cell of mesocarp 2. Oil cell
3. Stone cells of endocarp (3-1 in top view, 3-2 in lateral view)
4. Prisms 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

Linoleic acid standard solution

Weigh 1.5 mg of linoleic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and formic acid (7:2:0.2, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol. Sonicate (150 W) the mixture for 30 min. Filter the solution. Make appropriate dilution where necessary.

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 linoleic acid standard solution (0.5 µL) and the test solution (1.5 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 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 105ºC (about 2-4 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

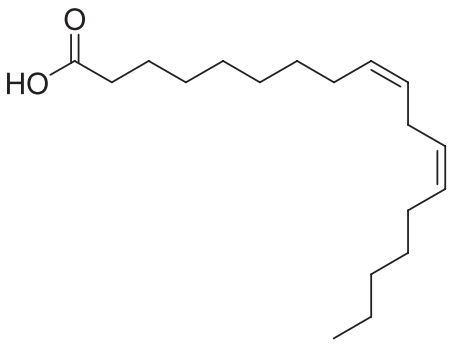

Figure 4 Chemical structure of linoleic acid

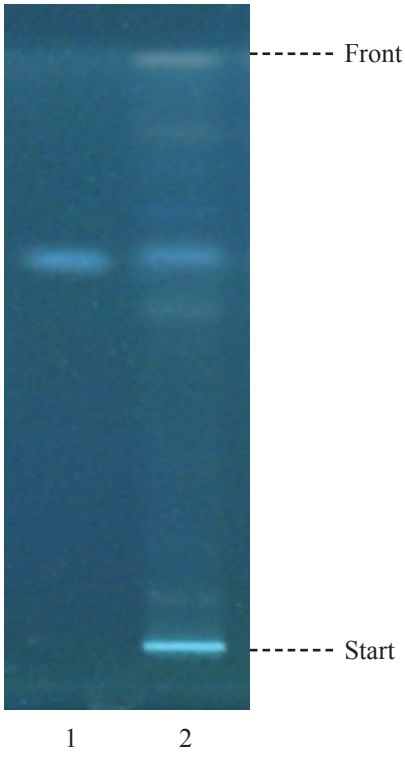

Figure 5 A reference HPTLC chromatogram of Litseae Fructus extract observed under UV light (366 nm) after staining

1. Linoleic acid 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 linoleic acid (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

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

Weigh 2.0 mg of linoleic acid CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (200 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.01% Acetic acid in acetonitrile (%, v/v)	0.01% Acetic acid (%, v/v)	Elution
0 – 42	30 → 90	70 → 10	linear gradient
42 – 60	90	10	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of linoleic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of linoleic acid should not be more than 5.0%; the RSD of the retention time of linoleic acid peak should not be more than 2.0%; the column efficiency determined from linoleic acid peak should not be less than 200000 theoretical plates.

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. 6).

Procedure

Separately inject linoleic acid Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of linoleic acid peak in the chromatogram of linoleic acid Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify linoleic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of linoleic acid Std-FP. The retention times of linoleic acid peaks from the two chromatograms 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 Litseae Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Litseae Fructus extract

Peak No.	RRT	Acceptable Range
1	0.53	± 0.03
2	0.71	± 0.03
3	0.91	± 0.03
4 (marker, linoleic acid)	1.00	-
5	1.14	± 0.03

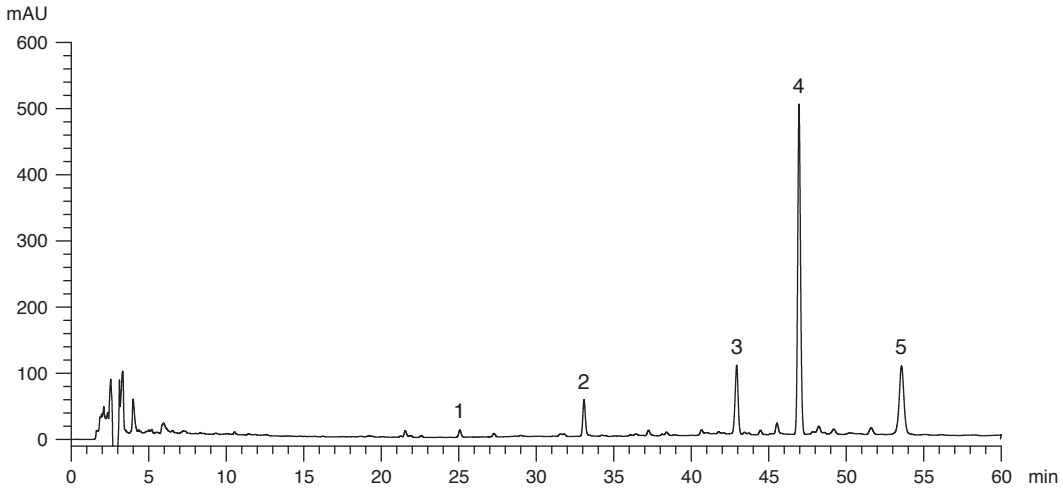

Figure 6 A reference fingerprint chromatogram of Litseae Fructus extract

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 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 – Aflatoxins *(Appendix VII)*: meet the requirements.

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

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 7.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 4.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution

Linoleic acid standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of linoleic acid CRS and dissolve in 10 mL of ethanol.

Linoleic acid standard solution for assay, Std-AS

Measure accurately the volume of the linoleic acid Std-Stock, dilute with ethanol to produce a series of solutions of 10, 20, 80, 200, 600 mg/L for linoleic acid.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (200 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% acetic acid and acetonitrile (14:86, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of linoleic acid Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of linoleic acid should not be more than 5.0%; the RSD of the retention time of linoleic acid peak should not be more than 2.0%; the column efficiency determined from linoleic acid peak should not be less than 10000 theoretical plates.

The *R* value between linoleic acid 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 linoleic acid Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of linoleic acid against the corresponding concentrations of linoleic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify linoleic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of linoleic acid Std-AS. The retention times of linoleic acid 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 linoleic acid in the test solution, and calculate the percentage content of linoleic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.32% of linoleic acid ($C_{18}H_{32}O_2$), calculated with reference to the dried substance.