Ricini Semen

Figure 1 A photograph of Ricini Semen

A. Ricini Semen B. Magnified seeds C. Magnified seed without testa

1. NAMES

Official Name: Ricini Semen

Chinese Name: 蓖麻子

Chinese Phonetic Name: Bimazi

2. SOURCE

Ricini Semen is the dried ripe seed of *Ricinus communis* L. (Euphorbiaceae). The ripe fruit is collected in autumn, dried under the sun, pericarp removed, then the seeds gathered to obtain Ricini Semen.

3. DESCRIPTION

Ellipsoid to ovoid, slightly flattened, 0.8-1.8 cm long, 0.5-1.2 cm wide. Externally smooth, with greyish-white and blackish-brown or yellowish-brown and reddish-brown alternate striations. One side relatively flat and the other side relatively convex, the flatter side with a protuberant raphe; one end with a protuberant greyish-white or pale brown caruncle. Testa thin and fragile; endosperm plump, white and oily; cotyledons 2, thin. Odour slight. Toxic, use with precaution (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis of testa consist of 1 layer of rectangular cells, covered with cuticle. Parenchyma of testa consists of 3-4 layers of cells, located underneath the epidermal cells of testa. Palisade cells of testa reddish-brown, with thickened cell wall, lignified. Perisperm consists of several layers of cells, shrunken, distributed with numerous crystals of calcium oxalate. Endosperm cells and cotyledon cells polygonal, containing oil droplets and aleurone granules. Raphe vascular bundle occurring underneath epidermal cells of testa (Fig. 2).

Powder

Colour greyish-yellow to yellowish-brown. Epidermal cells of testa brown or white, polygonal, walls beaded-thickened. Palisade cells of testa reddish-brown, polygonal in surface view, long cylindrical in lateral view, arranged orderly, cell walls thick, lignified, pits fine and dense, lumens contain reddish-brown contents. Perisperm cells with indistinct walls, densely covered with crystals of calcium oxalate in spherical, elliptic or rosette aggregates, 4-23 μm in diameter; polychromatic under the polarized microscope. Endosperm cells subpolygonal, containing aleurone grains and oil droplets (Fig. 3).

Footnotes: The powder should treat with petroleum ether (please refer to remarks for detail procedures) to remove excess oil, then followed by the routine procedure (Appendix III) to observe the characteristic features.

Remarks:

The following procedures should be carried out in fume hood.

1. Put 3 g of powder of Ricini Semen into a 100-mL conical flask.
2. Add 50 mL petroleum ether.
3. Cover the conical flask with aluminium foil, sonicate for 20 minutes. Set still for 5 minutes, pour out petroleum ether.
4. Repeat 2-3 for 2 times.
5. Filter the solution. Rinse the residue (powder) with petroleum ether for 2 times.
6. Let the residue dry. Grind the residue again if necessary.

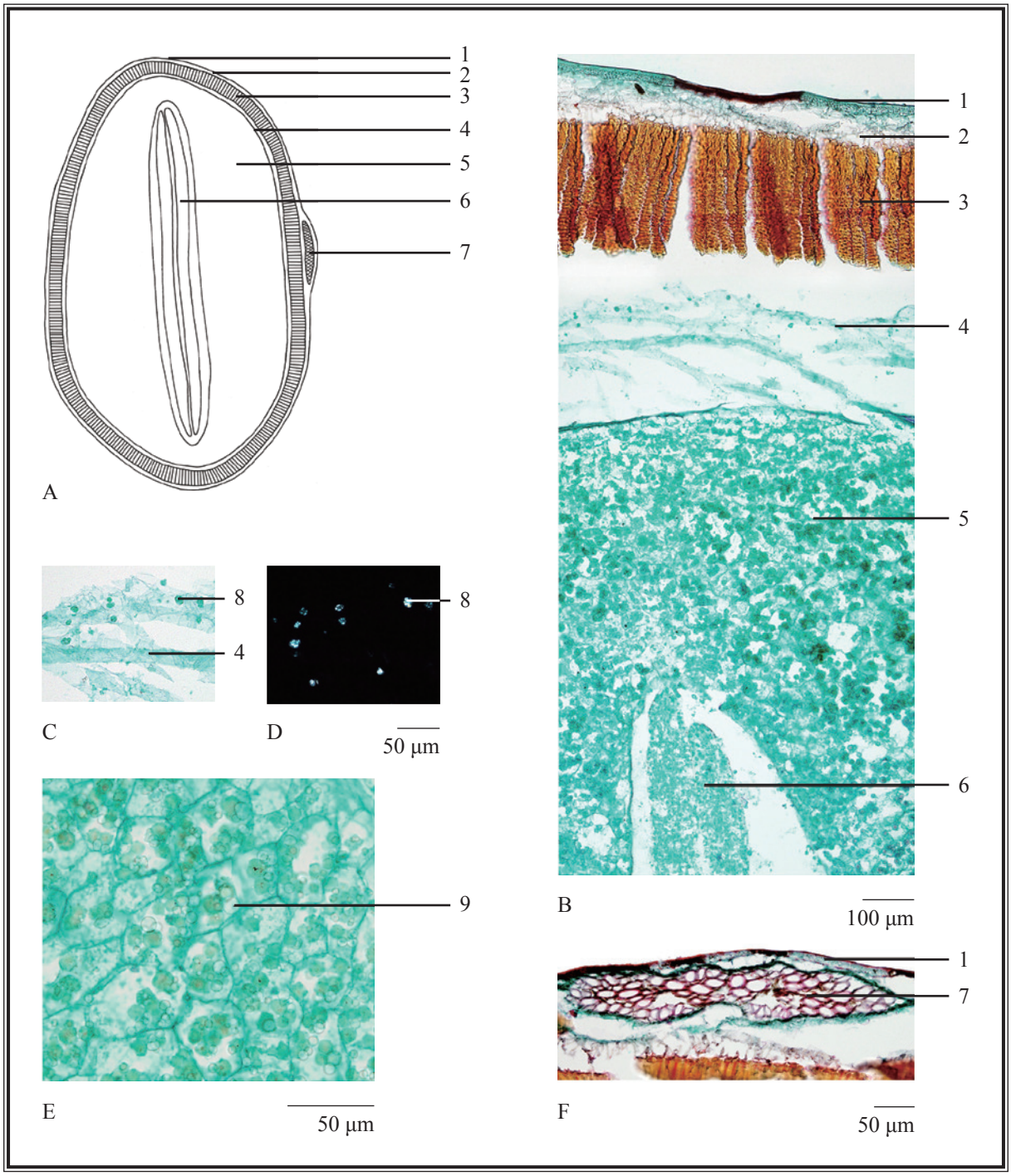

Figure 2 Microscopic features of transverse section of Ricini Semen

A. Sketch B. Section illustration C. Parenchymatous cells of testa
D. Parenchymatous cells of testa (under the polarized microscope) E. Endosperm F. Raphe vascular bundle

1. Epidermis of testa 2. Parenchymatous cells of testa 3. Palisade cells of testa 4. Perisperm
5. Endosperm 6. Cotyledon 7. Raphe vascular bundle 8. Crystal of calcium oxalate 9. Oil droplet

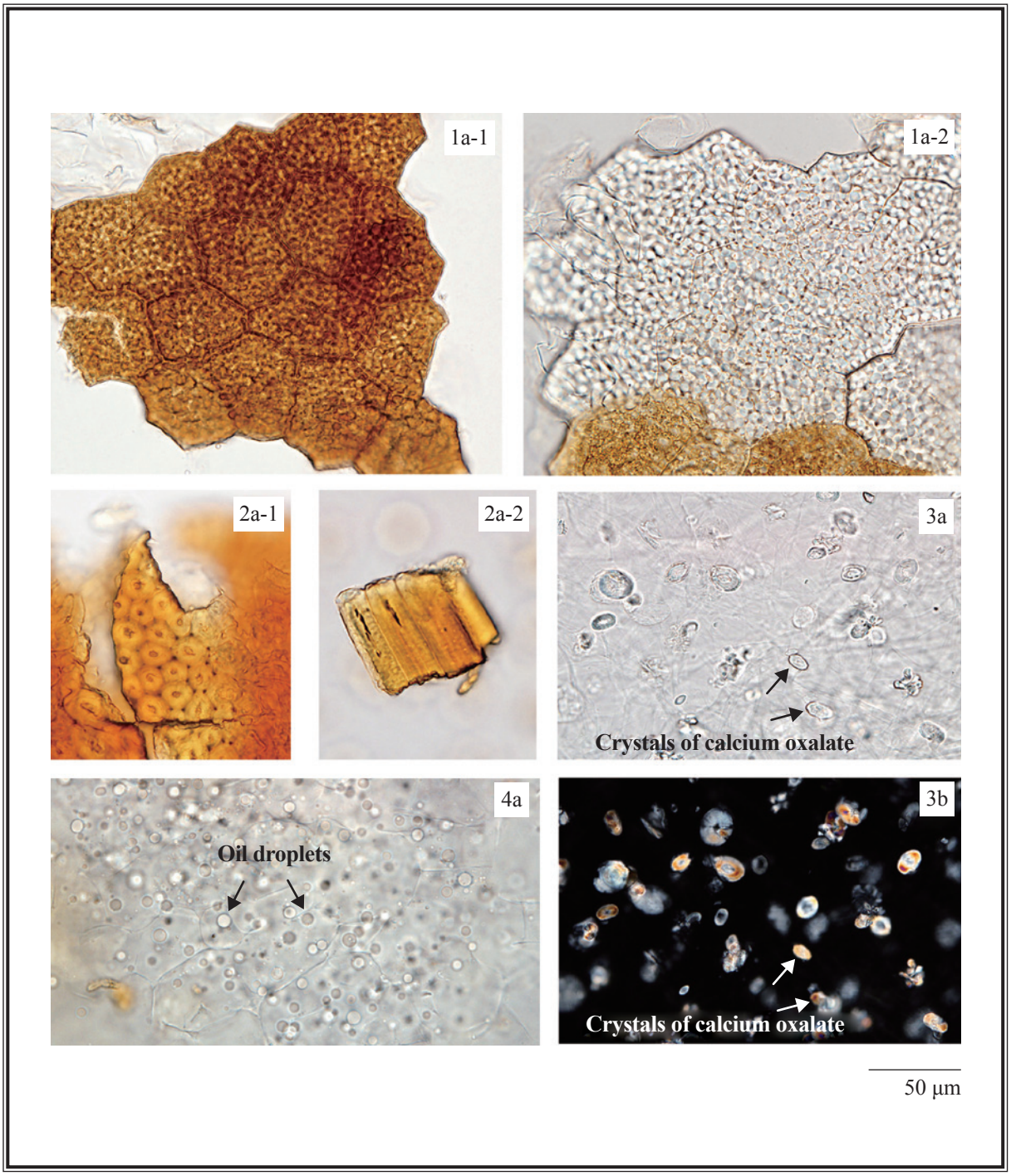

Figure 3 Microscopic features of powder of Ricini Semen

1. Epidermal cells of testa (1-1 brown, 1-2 white)
2. Palisade cells of testa (2-1 in surface view, 2-2 in lateral view)
3. Perisperm cells with crystals of calcium oxalate 4. Endosperm cells with oil droplets

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

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

Reagent

Cold ethanol

Store ethanol at 4ºC for 15 h.

Standard solution

Ricinoleic acid standard solution

Weigh 2.0 mg of ricinoleic acid CRS (Fig. 4) and dissolve in 2 mL of cold ethanol. Freshly prepare the standard solution.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC), ethyl acetate and formic acid (7:2:0.2, 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 0.1 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 25 mL of cold ethanol. Place the mixture in an ice bath for 30 min. Filter the mixture. Freshly prepare the test solution.

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 ricinoleic acid standard solution and the test solution (2 μL each) 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 105ºC until the spots or bands become visible (about 2-4 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

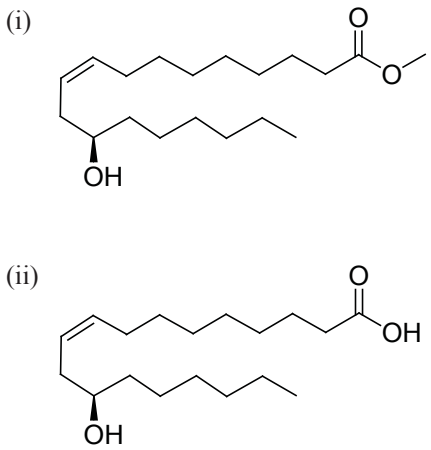

Figure 4 Chemical structures of (i) methyl ricinoleate and (ii) ricinoleic acid

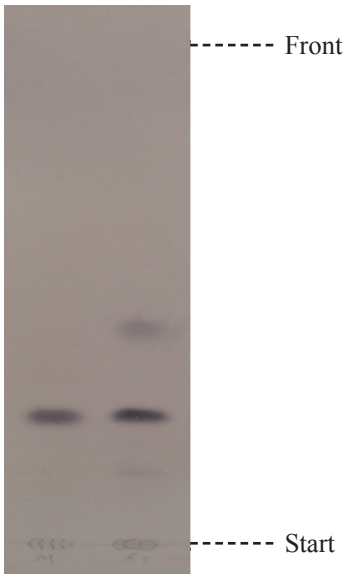

Figure 5 A reference HPTLC chromatogram of Ricini Semen extract observed under visible light after staining

1. Ricinoleic 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 ricinoleic acid (Fig. 5).

4.3 Gas Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

Methyl ricinoleate standard solution for fingerprinting, Std-FP (1000 mg/L)

Weigh 5.0 mg of methyl ricinoleate CRS (Fig. 4) and dissolve in 5 mL of diethyl ether.

Test solution

Weigh 0.5 g of the freshly powdered sample and place it in a 100-mL conical flask, then add 30 mL of *n*-hexane. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 10 mL of *n*-hexane. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 15 mL of methanol and 0.15 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of diethyl ether. Transfer the solution to a 250-mL separating funnel. Extract with 10 mL of water and collect the diethyl ether extract. Extract the aqueous layer for two times each with 30 mL of diethyl ether. Combine the diethyl ether extracts and add about 1.0 g of anhydrous sodium sulphate. Filter and transfer the filtrate to a 100-mL volumetric flask. Make up to the mark with diethyl ether. Filter through a 0.45-µm PTFE filter. Freshly prepare the test solution.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-WAX, 0.25 mm × 30 m) of which the internal wall is covered with polyethylene glycol in a layer about 0.25 µm thick. The injection temperature is at 280°C. The detector temperature is at 280°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 (°C)	Rate (°C/min)
0 – 30	180	-
30 – 38	180 → 220	5
38 – 68	220	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of methyl ricinoleate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of methyl ricinoleate should not be more than 5.0%; the RSD of the retention time of methyl ricinoleate peak should not be more than 2.0%; the column efficiency determined from methyl ricinoleate peak should not be less than 100000 theoretical plates.

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 methyl ricinoleate Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention time of methyl ricinoleate peak in the chromatogram of methyl ricinoleate Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify methyl ricinoleate peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of methyl ricinoleate Std-FP. The retention times of methyl ricinoleate 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 Ricini Semen extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (methyl palmitate)	0.14	± 0.03
2 (methyl stearate)	0.26	± 0.03
3 (methyl oleate)	0.27	± 0.03
4 (methyl linoleate)	0.32	± 0.03
5 (marker, methyl ricinoleate)	1.00	-

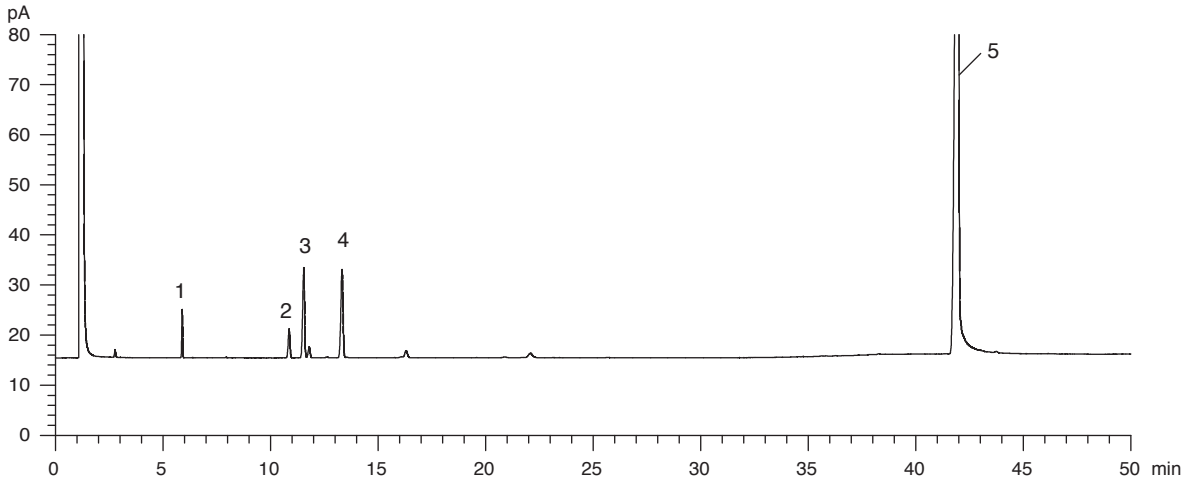

Figure 6 A reference GC fingerprint chromatogram of Ricini Semen extract

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 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 3.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 7.0%.

5.8 Acid Value *(Appendix XIV)*: not more than 35.0.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 7.0%.
Ethanol-soluble extractives (hot extraction method): not less than 28.0%.

7. ASSAY

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

Standard solution

Methyl ricinoleate standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 20.0 mg of methyl ricinoleate CRS and dissolve in 10 mL of diethyl ether.

Methyl ricinoleate standard solution for assay, Std-AS

Measure accurately the volume of the methyl ricinoleate Std-Stock, dilute with diethyl ether to produce a series of solutions of 50, 100, 200, 500, 800 mg/L for methyl ricinoleate.

Test solution

Weigh accurately 0.1 g of the freshly powdered sample and place it in a 100-mL conical flask, then add 30 mL of *n*-hexane. Sonicate (220 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 10 mL of *n*-hexane. Repeat the extraction for one more time. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 15 mL of methanol and 0.15 mL of hydrochloric acid. Reflux the mixture for 1.5 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 30 mL of diethyl ether. Transfer the solution to a 250-mL separating funnel. Extract with 10 mL of water and collect the diethyl ether extract. Extract the aqueous layer for two times each with 30 mL of diethyl ether. Combine the diethyl ether extracts and add about 1.0 g of anhydrous sodium sulphate. Filter and transfer the filtrate to a 100-mL volumetric flask. Make up to the mark with diethyl ether. Filter through a 0.45-µm PTFE filter. Freshly prepare the test solution.

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 280°C. The detector temperature is at 280°C. The split injection mode at a ratio of 100:1 is used. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C/min)
0 – 20	220	-
20 – 26	220 → 280	10
26 – 36	280	-

System suitability requirements

Perform at least five replicate injections, each using 1 µL of methyl ricinoleate Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of methyl ricinoleate should not be more than 5.0%; the RSD of the retention time of methyl ricinoleate peak should not be more than 2.0%; the column efficiency determined from methyl ricinoleate peak should not be less than 60000 theoretical plates.

The *R* value between methyl ricinoleate 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 methyl ricinoleate Std-AS (1 µL each) into the GC system and record the chromatograms. Plot the peak areas of methyl ricinoleate against the corresponding concentrations of methyl ricinoleate Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 1 µL of the test solution into the GC system and record the chromatogram. Identify methyl ricinoleate peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of methyl ricinoleate Std-AS. The retention times of methyl ricinoleate 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 methyl ricinoleate in the test solution, and calculate the percentage content of ricinoleic acid (the percentage content of methyl ricinoleate × 0.955) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 23% of ricinoleic acid ($C_{18}H_{34}O_3$), calculated with reference to the dried substance.