Allii Tuberosi Semen

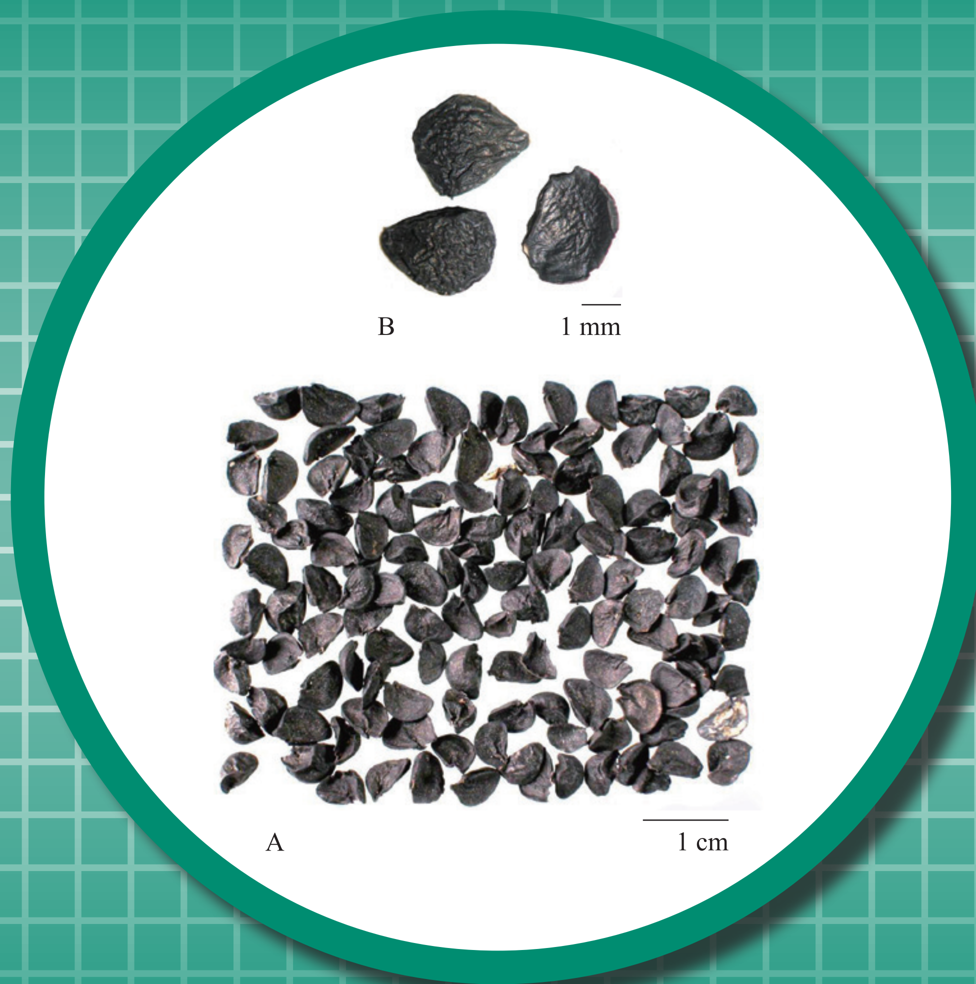

Figure 1 A photograph of Allii Tuberosi Semen

A. Allii Tuberosi Semen B. Magnified seeds

1. NAMES

Official Name: Allii Tuberosi Semen

Chinese Name: 韮菜子

Chinese Phonetic Name: Jiucaizi

2. SOURCE

Allii Tuberosi Semen is the dried ripe seed of *Allium tuberosum* Rottl. ex Spreng. (Liliaceae). The infructescence is collected in autumn when the fruit is ripe, dried under the sun, and gathered the seeds, foreign matter removed to obtain Allii Tuberosi Semen.

3. DESCRIPTION

Semi-spheroidal to semi-ovoid, slightly flattened, 2-4 mm long, 1.5-3 mm wide. Externally black, one side protuberant, rough, with finely and densely reticulate wrinkles; the other side slightly dented, wrinkles relatively indistinct. Apex obtuse, base slightly acute, with a protuberant and dotted hilum. Texture hard. Odour characteristic; taste slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, cells slightly flattened with thickened wall, the outer wall covered with cuticle, containing dark brown substances. Parenchyma of testa contains several layers of brownish-yellow cells, underneath the epidermis, arranged in crisscross. Endosperm occupies major portion of the seed, endosperm parenchymatous cells large, arranged densely, filling with aleurone grains and oil droplets. The embryo hook-liked, separately located near the upper and lower end of the seed in the transverse section, embryo cells arranged densely, filling with aleurone grains (Fig. 2).

Powder

Colour greyish-black. Epidermal cells black or brownish-black, elongated stripe-shaped, polygonal, subrounded or irregular, 44-132 µm long, 13-45 µm wide, with reticulate striations. Endosperm cells large, subrounded, walls relatively thickened, filling with aleurone grains and oil droplets. Parenchymatous cells underneath the epidermis, brownish-yellow, arranged in crisscross pattern, relatively large, mostly broken. Embryo cells in groups, rectangular or square (Fig. 3).

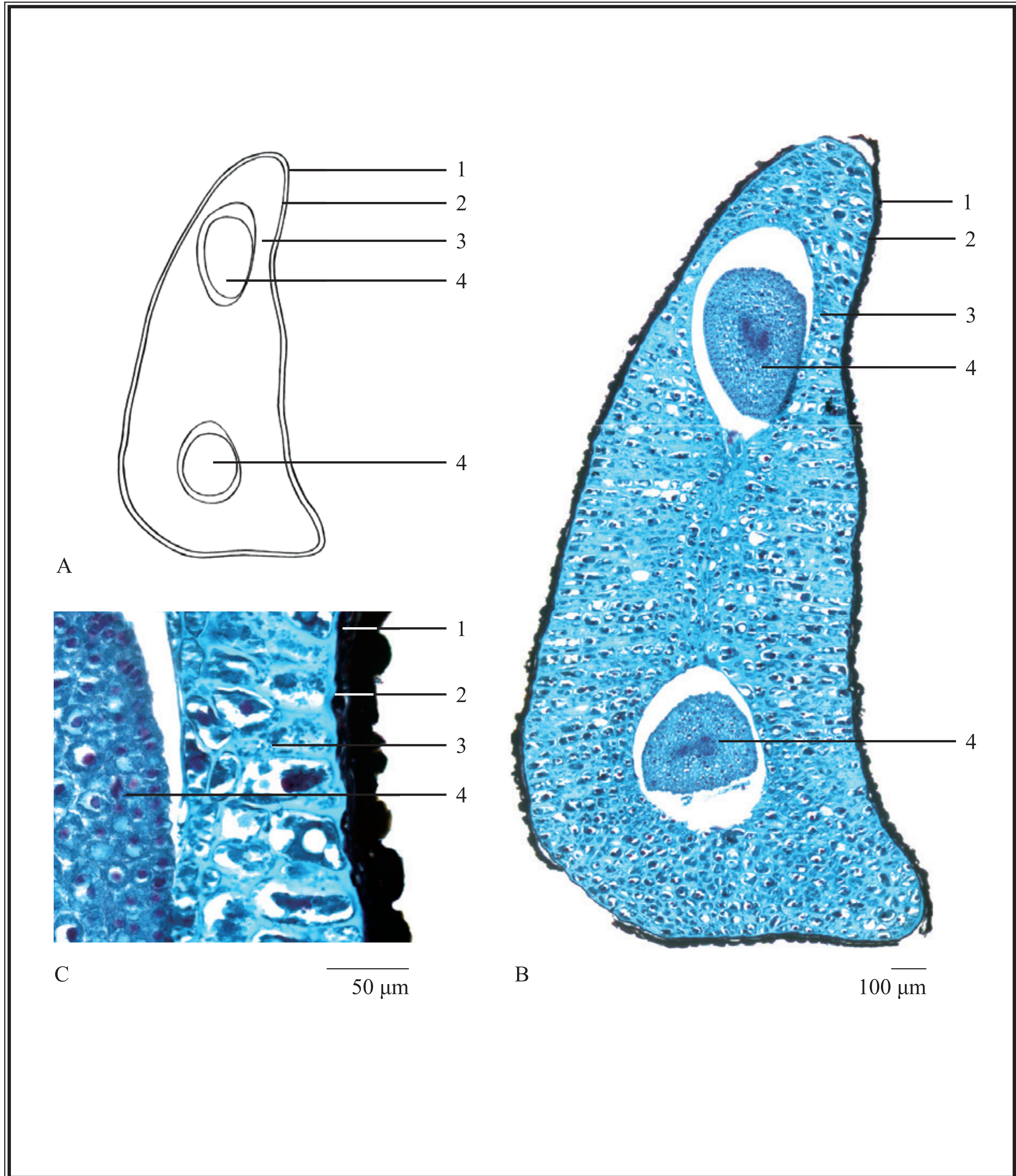

Figure 2 Microscopic features of transverse section of Allii Tuberosi Semen

A. Sketch B. Section illustration C. Epidermis, embryo and endosperm cells

1. Epidermis 2. Parenchyma of testa 3. Endosperm 4. Embryo

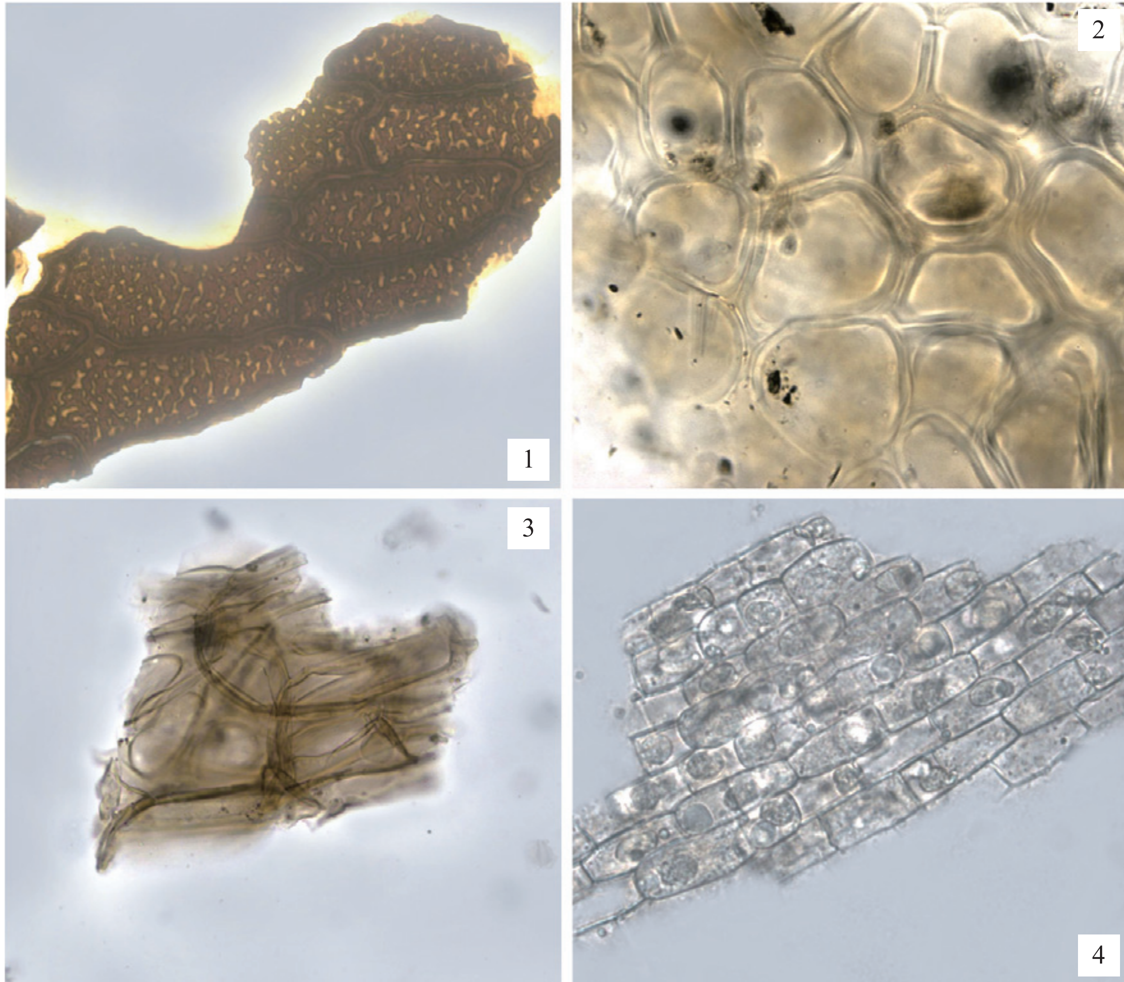

Figure 3 Microscopic features of powder of Allii Tuberosi Semen (under the light microscope)

1. Epidermal cells 2. Endosperm cells 3. Parenchymatous cells of testa 4. Embryo cells

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

Standard solution

Linoleic acid standard solution

Weigh 0.3 mg of linoleic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C) and acetone (2:1, v/v).

Spray reagent

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

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol. Sonicate (100 W) the mixture for 20 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 linoleic acid 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 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

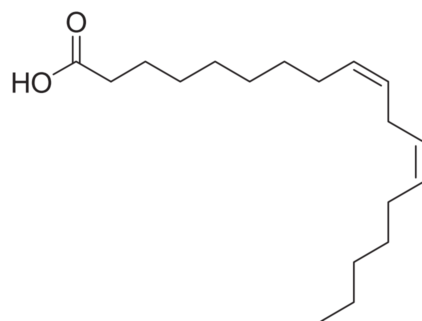

Figure 4 Chemical structure of linoleic acid

Figure 5 A reference HPTLC chromatogram of Allii Tuberosi Semen 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 (700 mg/L)

Weigh 7.0 mg of linoleic acid CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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)	Acetonitrile (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 40	20 → 100	80 → 0	linear gradient
40 – 50	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 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 400000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject linoleic acid Std-FP and the test solution (10 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 three 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 three characteristic peaks of Allii Tuberosi Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Allii Tuberosi Semen extract

Peak No.	RRT	Acceptable Range
1	0.73	± 0.03
2 (marker, linoleic acid)	1.00	-
3	1.08	± 0.03

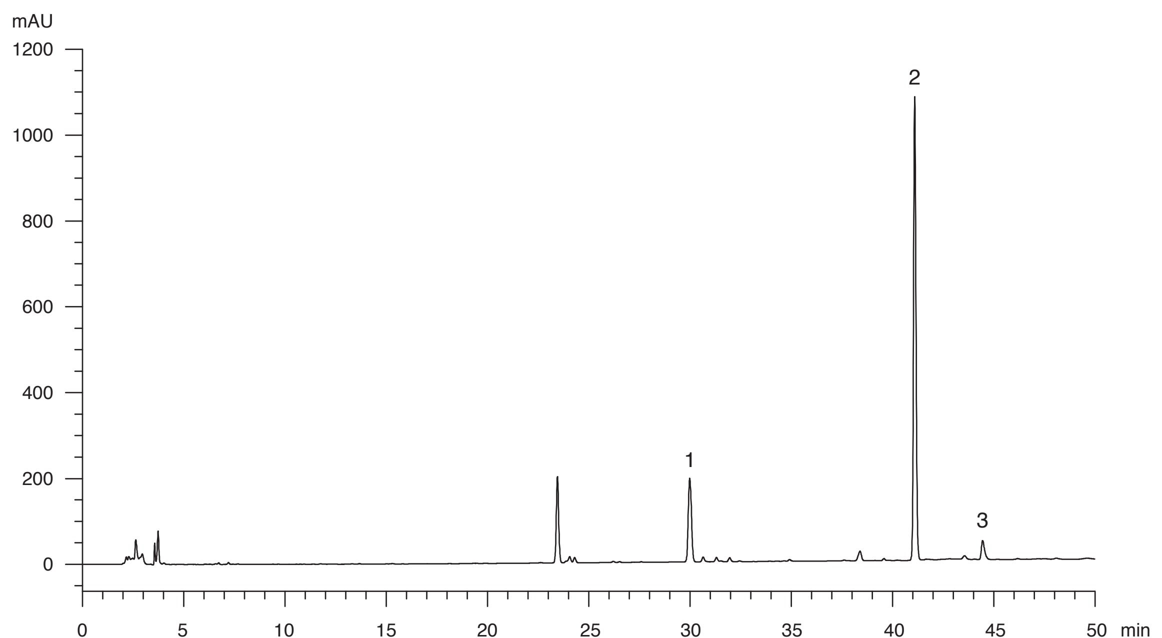

Figure 6 A reference fingerprint chromatogram of Allii Tuberosi Semen extract

For positive identification, the sample must give the above three 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 *(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 5.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 15.0%.

7. ASSAY

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

Standard solution

Linoleic acid standard stock solution, Std-Stock (3500 mg/L)

Weigh accurately 7.0 mg of linoleic acid CRS and dissolve in 2 mL of methanol.

Linoleic acid standard solution for assay, Std-AS

Measure accurately the volume of the linoleic acid Std-Stock, dilute with methanol to produce a series of solutions of 35, 70, 175, 262.5, 525 mg/L for linoleic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 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 0.2% phosphoric acid and acetonitrile (17:83, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of linoleic acid Std-AS (175 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 15000 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 (10 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 10 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 2.2% of linoleic acid ($C_{18}H_{32}O_2$), calculated with reference to the dried substance.