Lini Semen

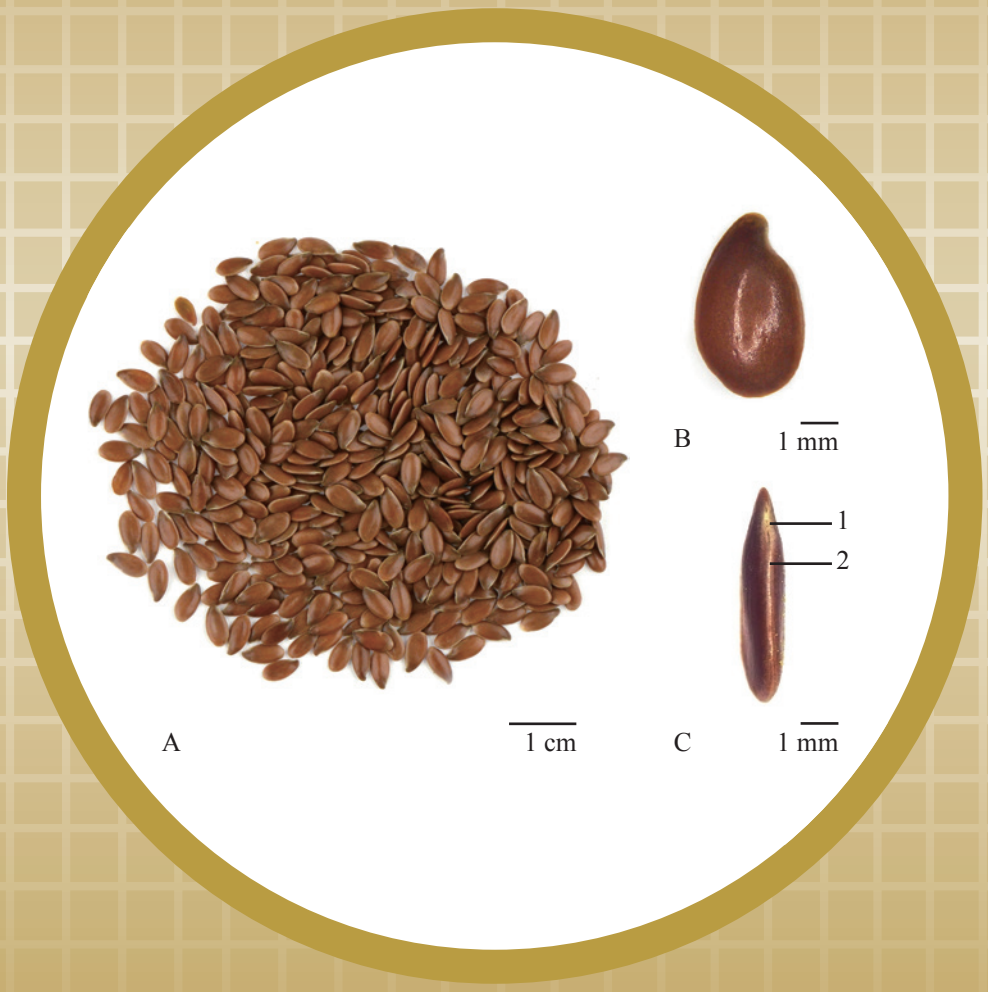

Figure 1 A photograph of Lini Semen

A. Lini Semen

B. Magnified image of seed

C. Magnified image of hilum (1) and raphe (2)

1. NAMES

Official Name: Lini Semen

Chinese Name: 亞麻子

Chinese Phonetic Name: Yamazi

2. SOURCE

Lini Semen is the dried ripe seed of *Linum usitatissimum* L. (Linaceae). The plant is collected in autumn when the seed is ripe, the harvested plant dried under the sun, the seeds tapped out, foreign matter removed, then the seeds are gathered and dried under the sun to obtain Lini Semen.

3. DESCRIPTION

Ovoid, flat, 2.3-5.9 mm long, 1.6-2.9 mm wide, 0.7-1.3 mm thick. Externally brown to reddish-brown, smooth and lustrous. One end acuminate and slightly oblique, the other end obtuse, with raphe and dented hilum on the acuminate end. Testa and endosperm thin, cotyledons 2, yellowish-white, oily. Odour slight, slightly flavoured and viscous on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Epidermis of testa consists of 1 layer of rectangular cells with mucilaginous walls. Hypodermis consists of 1 layer of cells, walls slightly thickened. Sclerenchymatous cells layer consists of 1 layer of densely arranged cells, walls thickened. Parenchymatous cells layer consists of collapsed cells, boundaries indistinct. Pigment layer consists of 1 layer of flattened cells filled with pigment masses. Endosperm consists of several layers of polygonal cells filled with aleurone grains. Cotyledon cells relatively small, filled with aleurone grains (Fig. 2).

Powder

Colour yellowish-brown to pale brown. Pigment cells pale yellow to yellow, square, rectangular or polygonal in surface view, 9-49 μm in diameter, anticlinal walls finely beaded, always containing yellowish-brown, brown or reddish-brown masses. Pigment masses yellowish-brown, brown or reddish-brown, square, rectangular or polygonal, edges usually finely serrated. Epidermal cells of testa large, polygonal in surface view, 16-69 μm in diameter, with mucilaginous walls. Sclerenchymatous cells pale yellow to yellow, strip-shaped, 4-23 μm in diameter, walls thickened or slightly thickened, with fine and dense pits; yellow or white under the polarized microscope. Hypodermal cells colourless to pale yellow, subpolygonal to subrounded in surface view, walls slightly thickened. Endosperm cells polygonal to subpolygonal, wall slightly thickened, filled with aleurone grains and oil droplets. Cotyledon cells polygonal to subpolygonal, relatively small, filled with aleurone grains and oil droplets (Fig. 3).

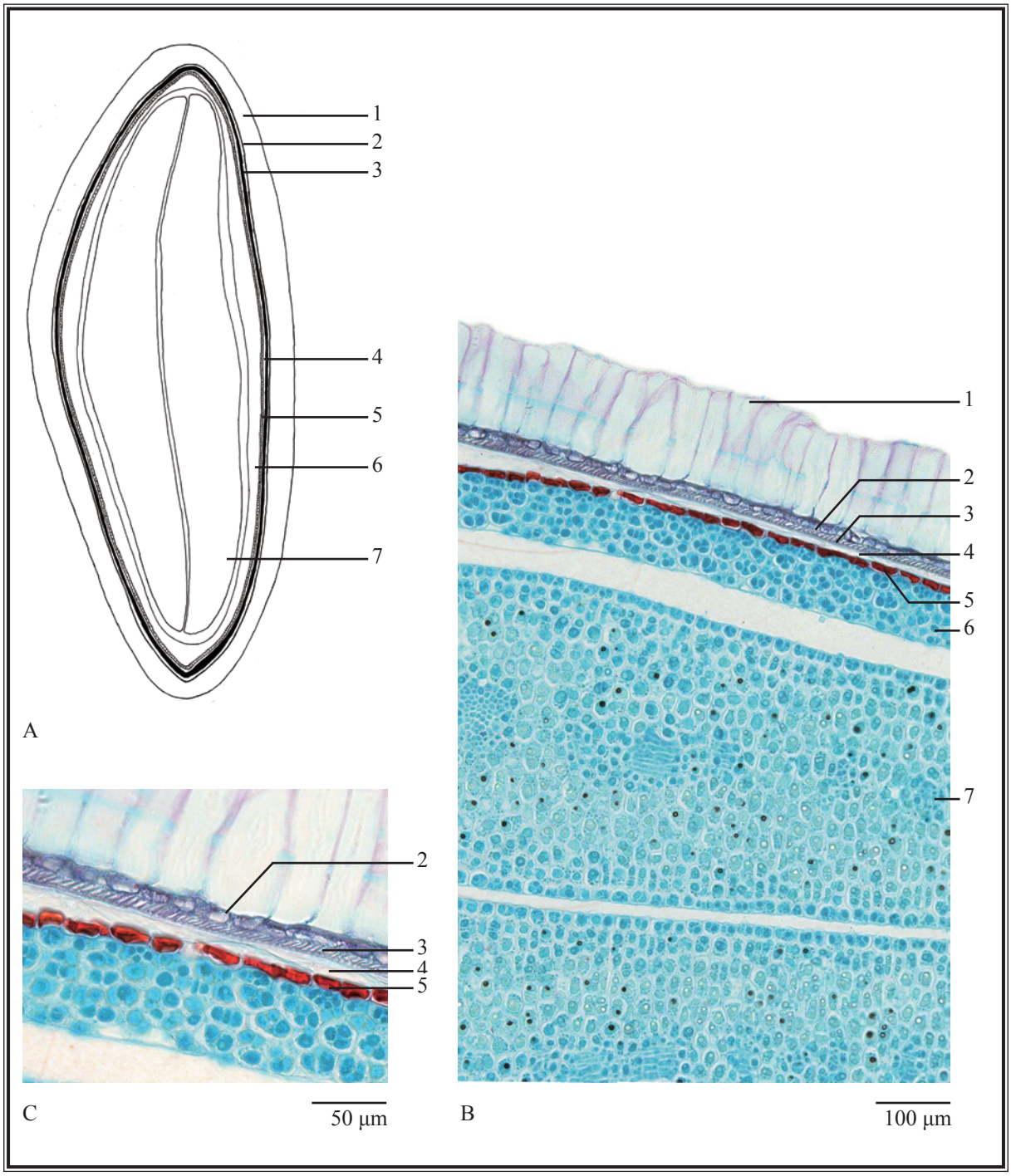

Figure 2 Microscopic features of transverse section of Lini Semen

A. Sketch B. Section illustration C. Magnified image of testa

1. Epidermis of testa 2. Hypodermis 3. Sclerenchymatous cell layer
4. Parenchymatous cell layer 5. Pigment layer 6. Endosperm 7. Cotyledon

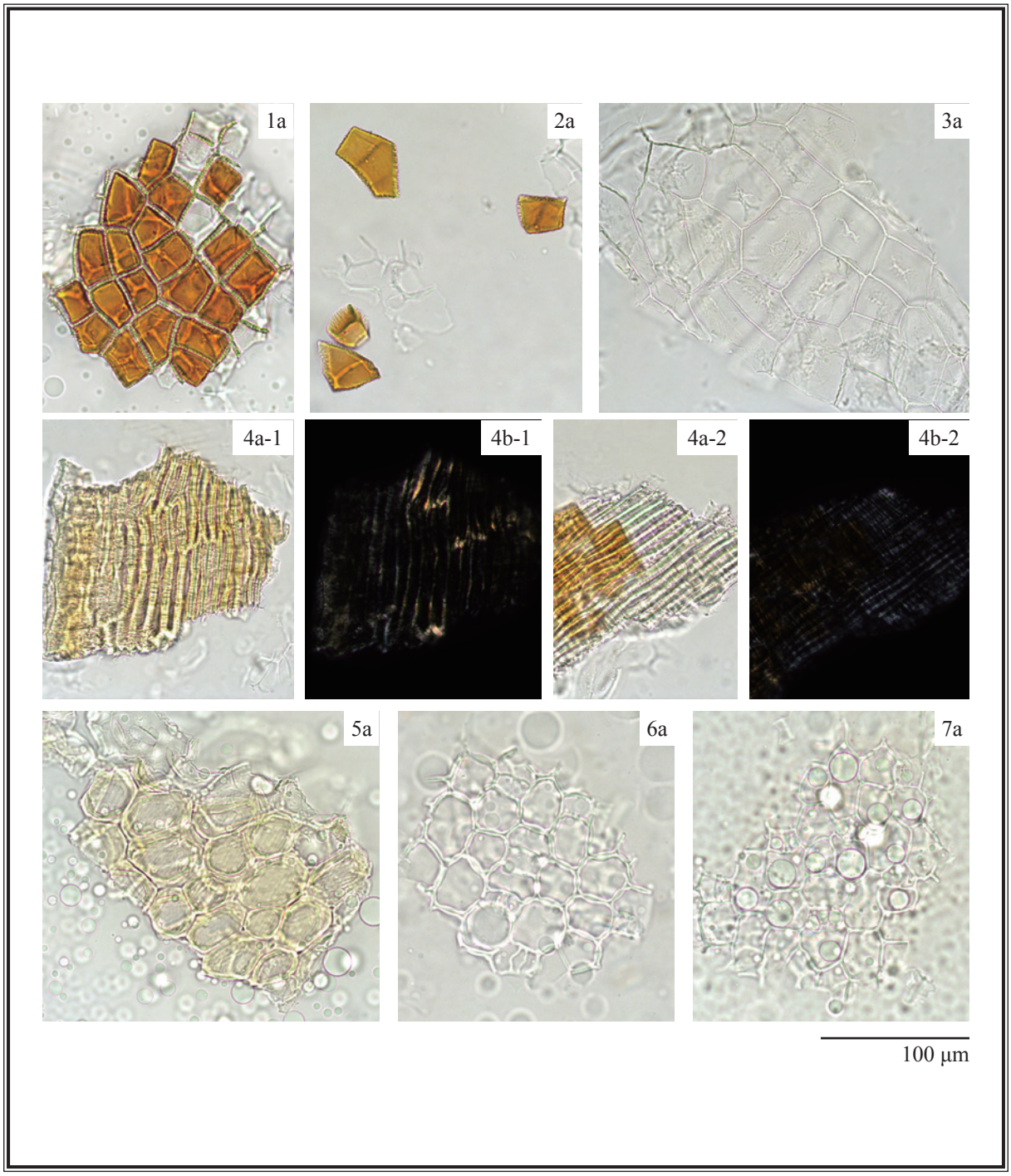

Figure 3 Microscopic features of powder of Lini Semen

1. Pigment cells 2. Pigment masses 3. Epidermal mucilage cells of testa
4. Sclerenchymatous cells (4-1 walls thickened, 4-2 walls slightly thickened)
5. Hypodermal cells 6. Endosperm cells 7. Cotyledon cells

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

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

Standard solutions

Linoleic acid standard solution

Weigh 0.2 mg of linoleic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

α-Linolenic acid standard solution

Weigh 0.2 mg of α-linolenic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of acetone, glacial acetic acid and dichloromethane (5:4:2, v/v).

Spray reagent

Add slowly 5 mL of sulphuric acid to 95 mL of ethanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of *n*-hexane. Sonicate (300 W) the mixture in a water bath at about 60ºC for 1 h. 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 5 mL of isopropanol. Filter through a 0.45-μm PTFE filter.

Procedure

Carry out the method by using a HPTLC RP-18 F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately linoleic acid standard solution (3.5 μL), α-linolenic acid standard solution (2.5 μL) and the test solution (6 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 3 - 5 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

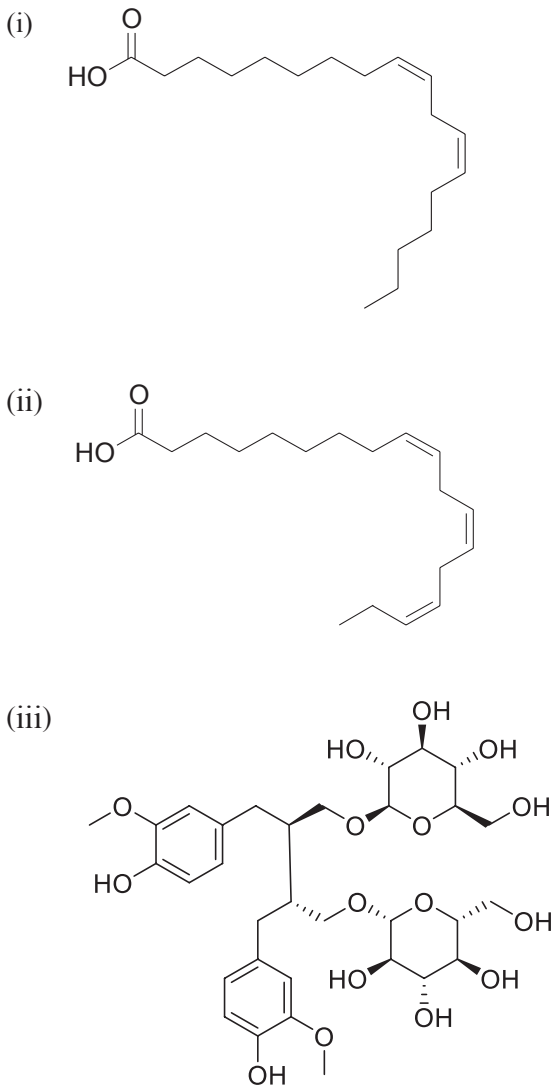

Figure 4 Chemical structures of (i) linoleic acid (ii) α-linolenic acid and (iii) secoisolariciresinol 1,4-diglucoside

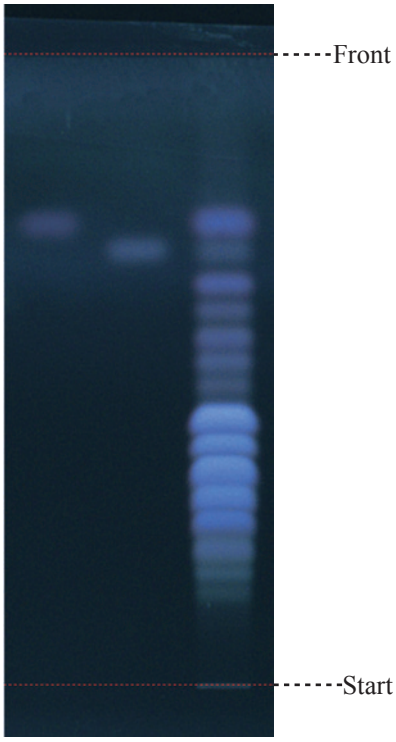

Figure 5 A reference HPTLC chromatogram of Lini Semen extract observed under UV light (366 nm) after staining

1. α-Linolenic acid standard solution 2. Linoleic acid standard solution 3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of linoleic acid and α-linolenic acid (Fig. 5).

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

Standard solutions

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

Weigh 0.3 mg of linoleic acid CRS and dissolve in 10 mL of ethanol.

α-Linolenic acid standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of α-linolenic acid CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of *n*-hexane. Sonicate (300 W) the mixture in a water bath at about 60ºC for 1 h. Centrifuge at

about 4000 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in isopropanol. Transfer the solution to a 25-mL of volumetric flask and make up to the mark with isopropanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (2.1 × 100 mm) packed with OS bonded silica gel (3.5 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile : Isopropanol (9:1, v/v) (%, v/v)	Elution
0 – 5	80 → 25	20 → 75	linear gradient
5 – 10	25	75	isocratic
10 – 18	25 → 0	75 → 100	linear gradient
18 – 40	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of linoleic acid Std-FP and α-linolenic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of linoleic acid and α-linolenic acid should not be more than 5.0%; the RSD of the retention times of linoleic acid and α-linolenic acid peaks should not be more than 2.0%; the column efficiencies determined from linoleic acid and α-linolenic acid peaks should not be less than 32000 theoretical plates.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 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, α-linolenic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of linoleic acid and α-linolenic acid peaks in the chromatograms of linoleic acid Std-FP, α-linolenic acid Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the

test solution. Identify linoleic acid and α-linolenic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of linoleic acid Std-FP and α-linolenic acid Std-FP. The retention times of linoleic acid and α-linolenic acid 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 six characteristic peaks of Lini Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Lini Semen extract

Peak No.	RRT	Acceptable Range
1 (marker, α-linolenic acid)	1.00	-
2 (linoleic acid)	1.11	± 0.03
3	2.77	± 0.08
4	2.87	± 0.08
5	2.99	± 0.07
6	3.16	± 0.07

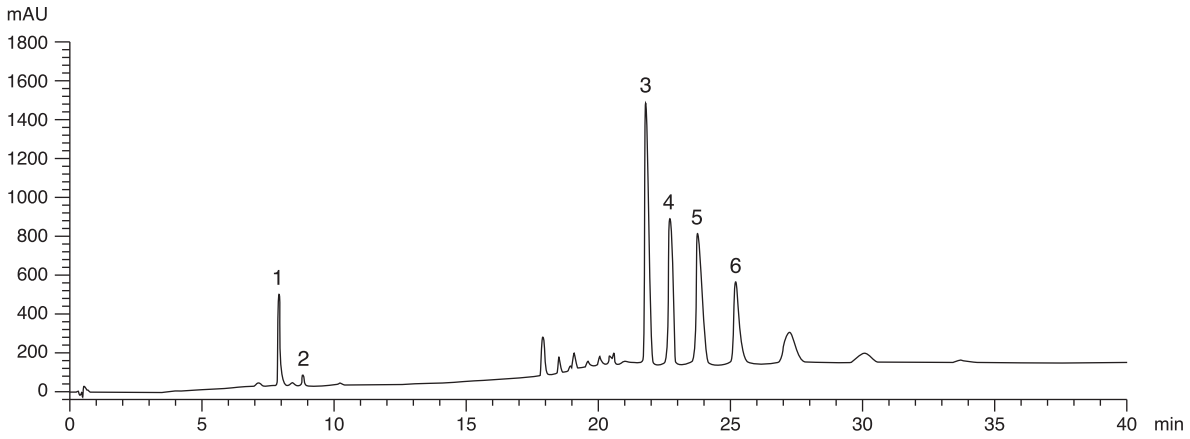

Figure 6 A reference fingerprint chromatogram of Lini Semen extract

For positive identification, the sample must give the above six 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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 8.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 16.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

7.1 Assay of linoleic acid and α-linolenic acid

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

Standard solution
Mixed linoleic acid and α-linolenic acid standard stock solution, Std-Stock (320 mg/L for linoleic acid and 400 mg/L for α-linolenic acid)
Weigh accurately 1.6 mg of linoleic acid CRS and 2.0 mg of α-linolenic acid CRS, and dissolve in 5 mL of ethanol.

Mixed linoleic acid and α-linolenic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed linoleic acid and α-linolenic acid Std-Stock, dilute with ethanol to produce a series of solutions of 5, 10, 20, 40, 80 mg/L for linoleic acid and 6.25, 12.5, 25, 50, 100 mg/L for α-linolenic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of *n*-hexane. Sonicate (300 W) the mixture in a water bath at about 60ºC for 1 h. Centrifuge at about 4000 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for two more times. (Collect the residue for the assay of secoisolariciresinol 1,4-diglucoside.) Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in isopropanol. Transfer the solution to a 25-mL of volumetric flask and make up to the mark with isopropanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (2.1 × 100 mm) packed with OS bonded silica gel (3.5 μm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile: Isopropanol (9:1, v/v) (%, v/v)	Elution
0 – 5	80 → 25	20 → 75	linear gradient
5 – 10	25	75	isocratic
10 – 18	25 → 0	75 → 100	linear gradient
18 – 40	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed linoleic acid and α-linolenic acid Std-AS (20 mg/L for linoleic acid and 25 mg/L for α-linolenic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of linoleic acid and α-linolenic acid should not be more than 5.0%; the RSD of the retention times of linoleic acid and α-linolenic acid peaks should not be more than 2.0%; the column efficiencies determined from linoleic acid and α-linolenic acid peaks should not be less than 32000 theoretical plates.

The *R* value between linoleic acid peak and the closest peak; and the *R* value between α-linolenic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curves

Inject a series of the mixed linoleic acid and α-linolenic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of linoleic acid and α-linolenic acid against the corresponding concentrations of the mixed linoleic acid and α-linolenic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify linoleic acid and α-linolenic acid peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed linoleic acid and α-linolenic acid Std-AS. The retention times of linoleic acid and α-linolenic acid 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 linoleic acid and α-linolenic acid in the test solution, and calculate the percentage contents of linoleic acid and α-linolenic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.56% of the total content of linoleic acid ($C_{18}H_{32}O_2$) and α-linolenic acid ($C_{18}H_{30}O_2$), calculated with reference to the dried substance.

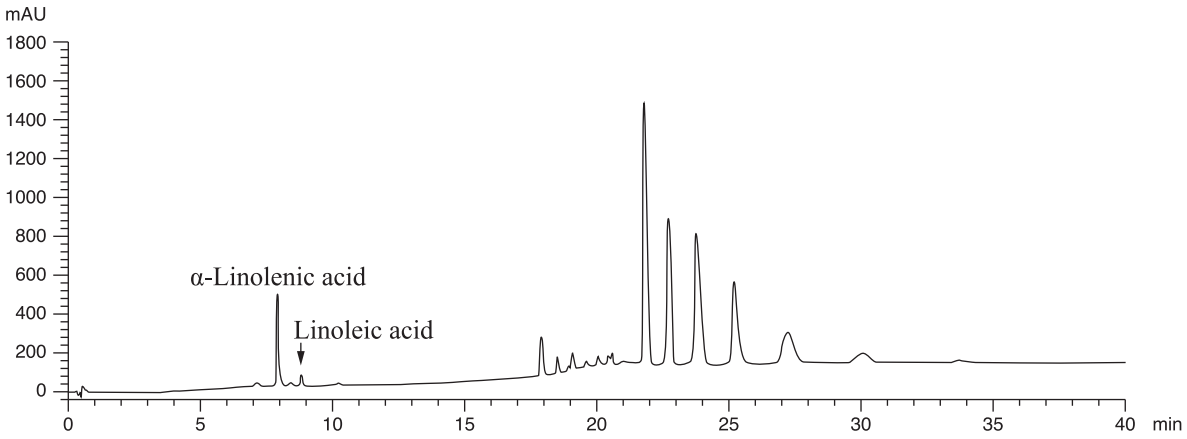

Figure 7 A reference assay chromatogram of linoleic acid and α-linolenic acid of Lini Semen extract

7.2 Assay of secoisolariciresinol 1,4-diglucoside

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

Standard solution

Secoisolariciresinol 1,4-diglucoside standard stock solution, Std-Stock (720 mg/L)

Weigh accurately 3.6 mg of secoisolariciresinol 1,4-diglucoside CRS (Fig. 4) and dissolve in 5 mL of methanol (70%).

Secoisolariciresinol 1,4-diglucoside standard solution for assay, Std-AS

Measure accurately the volume of the secoisolariciresinol 1,4-diglucoside Std-Stock, dilute with methanol (70%) to produce a series of solutions of 4.5, 9, 18, 36, 72 mg/L for secoisolariciresinol 1,4-diglucoside.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 50 mL of *n*-hexane. Sonicate (300 W) the mixture in a water bath at about 60ºC for 1 h. Centrifuge at about 4000 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for two more times. (Collect the filtrates for the assay of linoleic acid and α-linolenic acid.) Dry the residue for 2 h. Transfer the dried residue to a 50-mL conical flask, then add 20 mL of sodium hydroxide (4%, w/v) in methanol (70%). Cap the flask. Sonicate (300 W) the mixture in a water bath at about 60ºC for 1 h. Transfer the mixture to a 50-mL centrifuge tube and centrifuge at about 4000 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL conical flask. Repeat the extraction for one more time. Combine the filtrates and adjust the pH to 3 with hydrochloric acid (4.37%, w/v) in methanol (70%). Transfer the solution to a 100-mL volumetric flask and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 4) –

Table 4 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	85	15	isocratic
5 – 15	85 → 78	15 → 22	linear gradient
15 – 20	78 → 10	22 → 90	linear gradient
20 – 25	10	90	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of secoisolariciresinol 1,4-diglucoside Std-AS (18 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of secoisolariciresinol 1,4-diglucoside should not be more than 5.0%; the RSD of the retention time of secoisolariciresinol 1,4-diglucoside peak should not be more than 2.0%; the column efficiency determined from secoisolariciresinol 1,4-diglucoside peak should not be less than 25000 theoretical plates.

The *R* value between secoisolariciresinol 1,4-diglucoside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 8).

Calibration curve

Inject a series of secoisolariciresinol 1,4-diglucoside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of secoisolariciresinol 1,4-diglucoside against the corresponding concentrations of secoisolariciresinol 1,4-diglucoside 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 secoisolariciresinol 1,4-diglucoside peak (Fig. 8) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of secoisolariciresinol 1,4-diglucoside Std-AS. The retention times of secoisolariciresinol 1,4-diglucoside 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 secoisolariciresinol 1,4-diglucoside in the test solution, and calculate the percentage content of secoisolariciresinol 1,4-diglucoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.81% of secoisolariciresinol 1,4-diglucoside ($C_{32}H_{46}O_{16}$), calculated with reference to the dried substance.

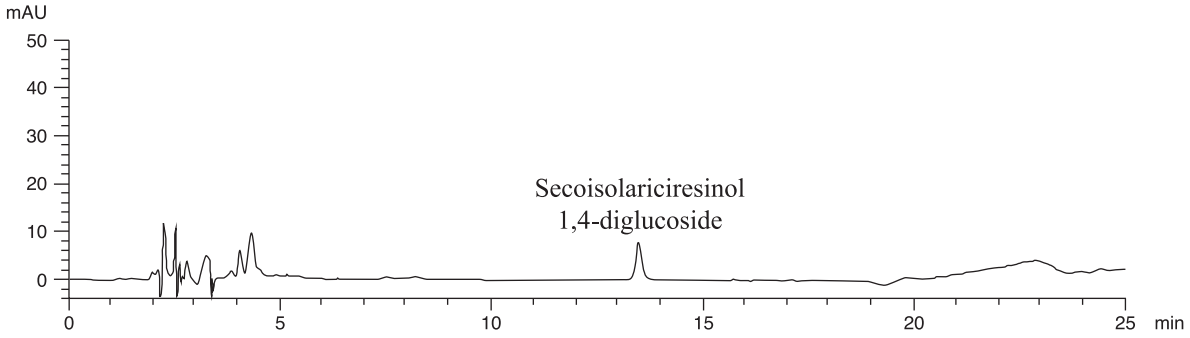

Figure 8 A reference assay chromatogram of secoisolariciresinol 1,4-diglucoside of Lini Semen extract