Tritici Levis Fructus

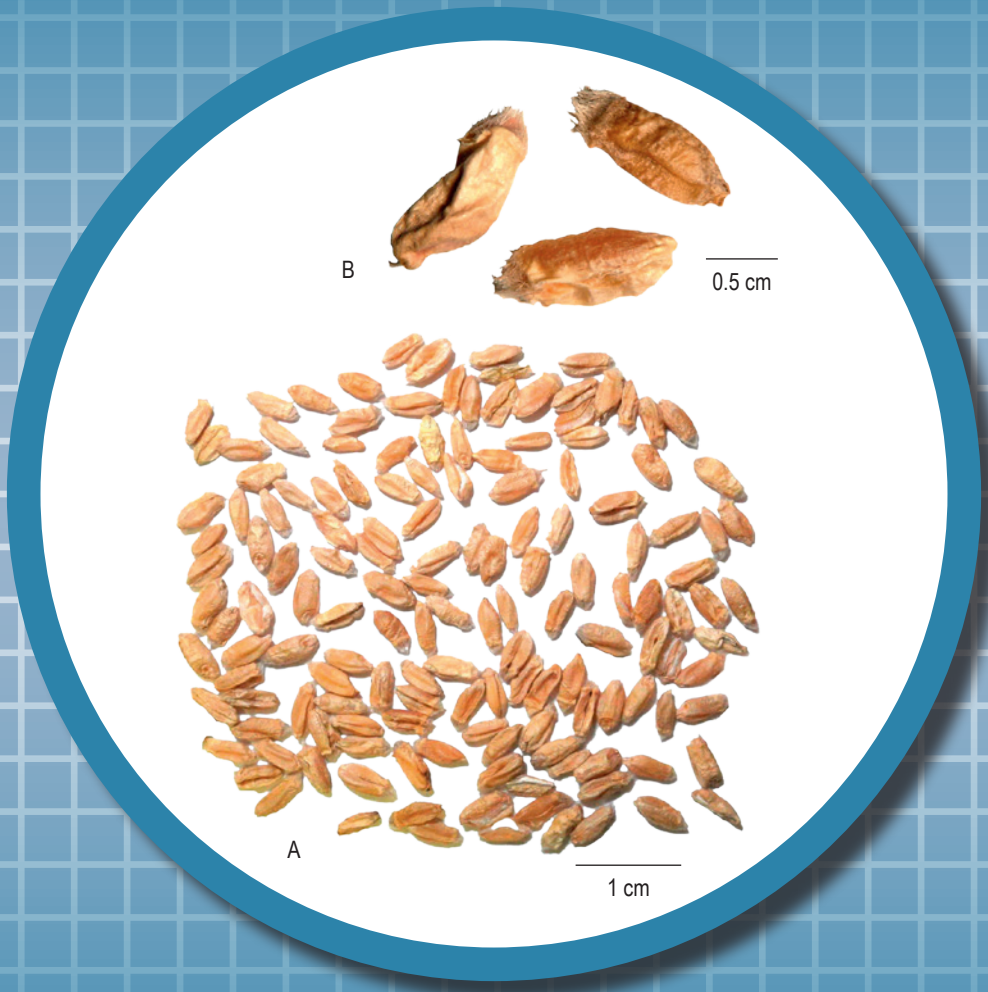

Figure 1 A photograph of Tritici Levis Fructus

A. Tritici Levis Fructus B. Magnified Tritici Levis Fructus

1. NAMES

Official Name: Tritici Levis Fructus

Chinese Name: 浮小麥

Chinese Phonetic Name: Fuxiaomai

2. SOURCE

Tritici Levis Fructus is the dried, shriveled and light caryopsis of *Triticum aestivum* L. (Poaceae). The caryopsis is collected around the summer solstice, the dust is sifted and rinsed with water, then dried under the sun to obtain Tritici Levis Fructus.

3. DESCRIPTION

Elongated, oblong-ellipsoid, both ends slightly acute, 4-8 mm long, 1.5-3 mm in diameter. Externally pale yellowish-brown to yellow, crumpled, with a deep crease on the centre part of abdomen; an indistinct embryo at the base of the back, with beard-like and pale yellow hairs at the apex on the dorsal side. Texture hard and fragile, easily broken. Fracture white to yellowish-white, starchy. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Pericarp fused with testa, the epidermal cells of the pericarp consist of 1 layer, with relatively thickened wall. Transverse cell 1 layer, oblong, arranged regularly, located inside the pericarp, with a relatively thickened wall. Aleurone layer located in the outermost side of endosperm, the cells square, filled with aleurone grains. Vascular tissue small, located deep in the crease. The embryo consists of parenchymatous cells. The endosperm embraces the embryo, filled with starch granules (Fig. 2).

Powder

Colour whitish. Starch granules varied in shape, globular to broadly ovoid, cucullate or reniform, 6-42 μm in diameter, hilum slit-shaped, striations indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-4 (even more) units. Cells of aleurone layer subsquare or subrounded, with intercellular space, wall slightly thickened, filled with aleurone grains. Transverse cells mostly in groups, arranged regularly, slender cylindrical, wall beaded-thickened. Non-glandular hairs unicellular, apex acute and pointed, base slightly constricted and rounded, 143-653 μm long. Epidermal cells of pericarp subrectangular, elongated or long polygonal in surface view, anticlinal wall beaded (Fig. 3).

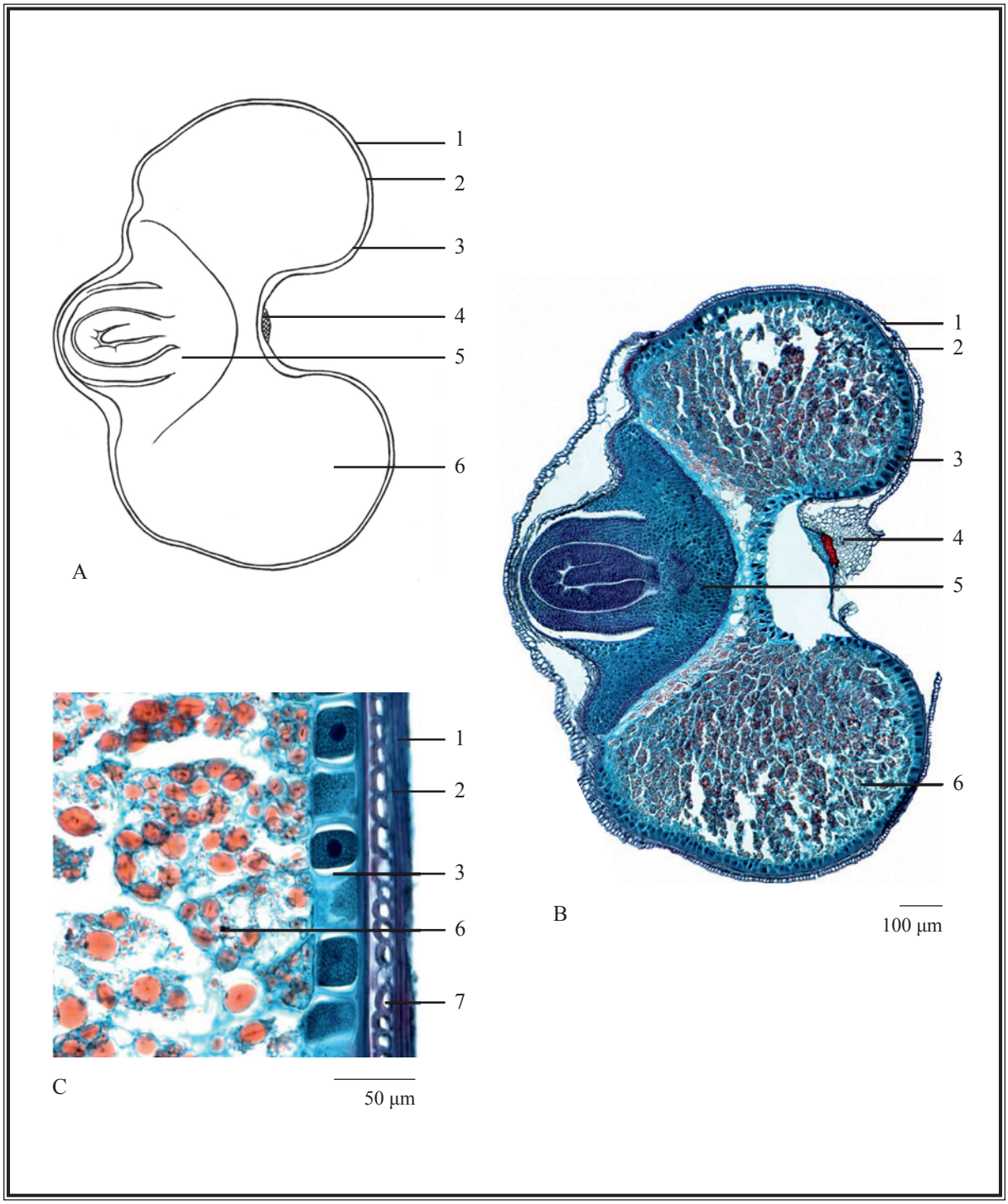

Figure 2 Microscopic features of transverse section of Tritici Levis Fructus

A. Sketch B. Section illustration C. Aleurone layer and endosperm

1. Pericarp 2. Testa 3. Aleurone layer 4. Vascular tissue 5. Embryo
6. Endosperm 7. Transverse cells

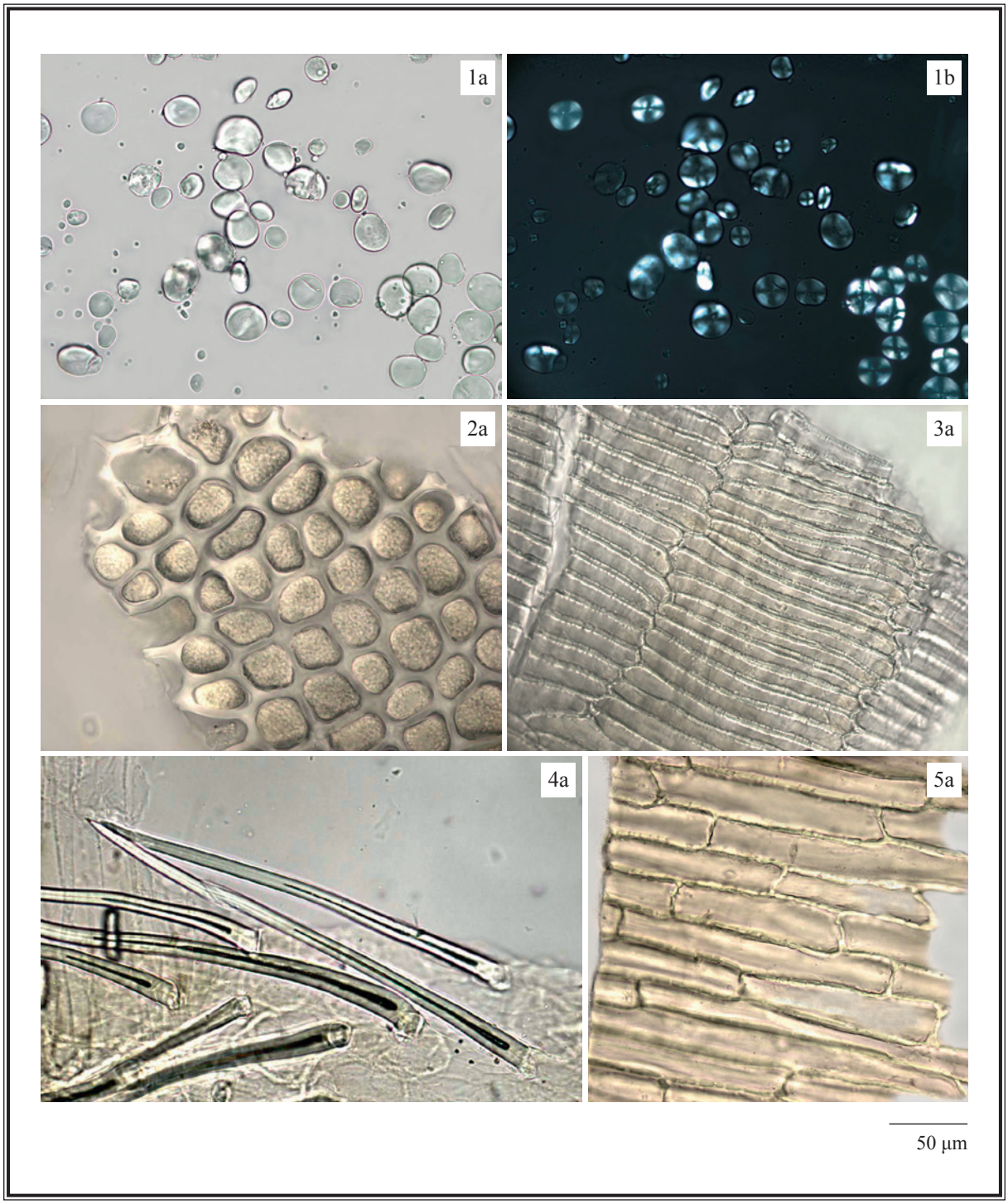

Figure 3 Microscopic features of powder of Tritici Levis Fructus

1. Starch granules 2. Aleurone layer cells (in surface view) 3. Transverse cells 4. Non-glandular hairs 5. Epidermal cells of pericarp

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

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

Standard solution

5-Heneicosylresorcinol standard solution

Weigh 0.1 mg of 5-heneicosylresorcinol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80˚C) and acetone (5:3, 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 10 mL of methanol. Sonicate (100 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Filter through a 0.45-μm nylon filter.

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 5-heneicosylresorcinol standard solution and the test solution (2 μL each) 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 105˚C 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).

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 5-heneicosylresorcinol.

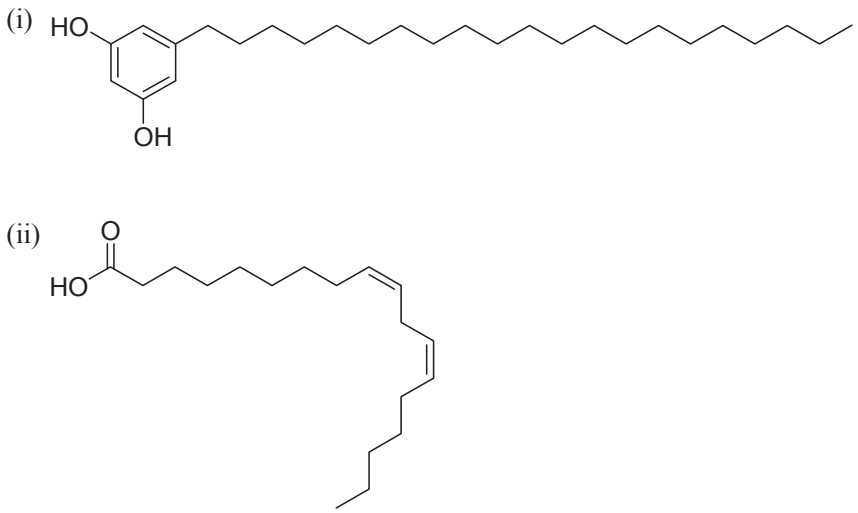

Figure 4 Chemical structures of (i) 5-heneicosylresorcinol and (ii) linoleic acid

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

Standard solutions

Linoleic acid standard solution for fingerprinting, Std-FP (350 mg/L)

Weigh 3.5 mg of linoleic acid CRS (Fig. 4) and dissolve in 10 mL of methanol.

5-Heneicosylresorcinol standard solution for fingerprinting, Std-FP (12 mg/L)

Weigh 0.6 mg of 5-heneicosylresorcinol CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 1.0 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)	0.1% Phosphoric acid (%, v/v)	Elution
0 – 25	30 → 100	70 → 0	linear gradient
25 – 60	100	0	isocratic

System suitability requirements

Perform at least five replicate injections, each using 20 µL of linoleic acid Std-FP and 5-heneicosylresorcinol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of linoleic acid and 5-heneicosylresorcinol should not be more than 5.0%; the RSD of the retention times of linoleic acid and 5-heneicosylresorcinol peaks should not be more than 2.0%; the column efficiencies determined from linoleic acid and 5-heneicosylresorcinol peaks should not be less than 160000 and 40000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject linoleic acid Std-FP, 5-heneicosylresorcinol Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of linoleic acid and 5-heneicosylresorcinol peaks in the chromatograms of linoleic acid Std-FP, 5-heneicosylresorcinol Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify linoleic acid and 5-heneicosylresorcinol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of linoleic acid Std-FP and 5-heneicosylresorcinol Std-FP. The retention times of linoleic acid and 5-heneicosylresorcinol 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 four characteristic peaks of Tritici Levis Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Tritici Levis Fructus extract

Peak No.	RRT	Acceptable Range
1	0.93	± 0.03
2 (marker, linoleic acid)	1.00	-
3	1.48	± 0.03
4 (5-heneicosylresorcinol)	1.81	± 0.05

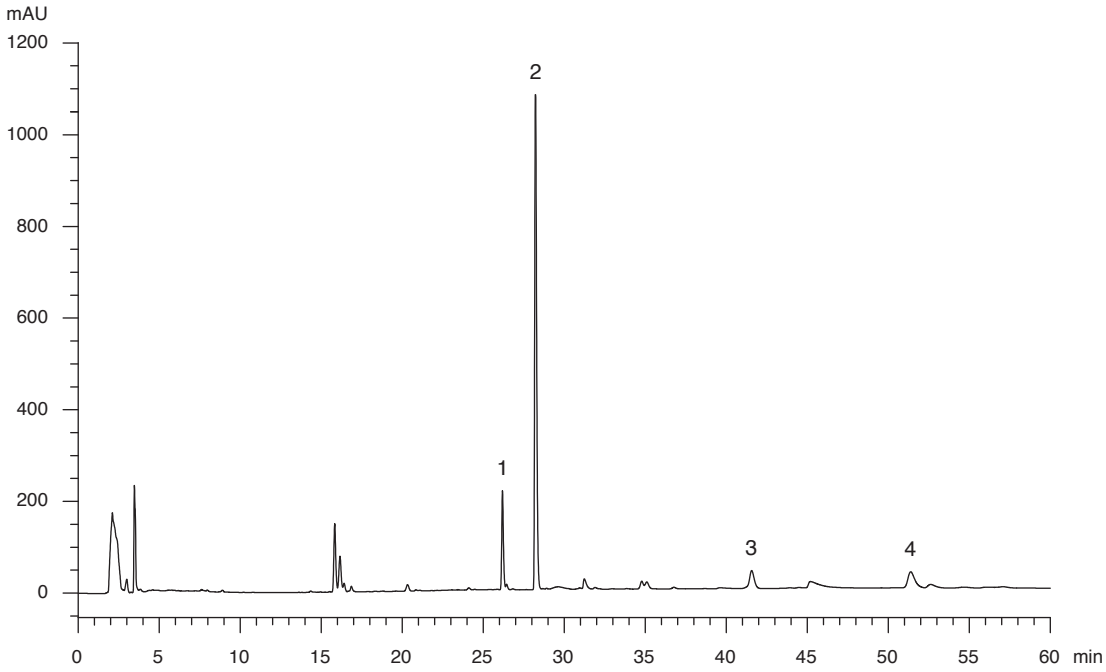

Figure 5 A reference fingerprint chromatogram of Tritici Levis Fructus extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 1.0%.

7. ASSAY

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

Standard solution

5-Heneicosylresorcinol standard stock solution, Std-Stock (565 mg/L)

Weigh accurately 11.3 mg of 5-heneicosylresorcinol CRS and dissolve in 20 mL of methanol.

5-Heneicosylresorcinol standard solution for assay, Std-AS

Measure accurately the volume of the 5-heneicosylresorcinol Std-Stock, dilute with methanol to produce a series of solutions of 2.83, 5.65, 14.13, 28.3, 56.5 mg/L for 5-heneicosylresorcinol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction for two more times. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask 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 methanol and water (96:4, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of 5-heneicosylresorcinol Std-AS (14.13 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 5-heneicosylresorcinol should not be more than 5.0%; the RSD of the retention time of 5-heneicosylresorcinol peak should not be more than 2.0%; the column efficiency determined from 5-heneicosylresorcinol peak should not be less than 10000 theoretical plates.

The *R* value between 5-heneicosylresorcinol 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 5-heneicosylresorcinol Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of 5-heneicosylresorcinol against the corresponding concentrations of 5-heneicosylresorcinol Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify 5-heneicosylresorcinol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 5-heneicosylresorcinol Std-AS. The retention times of 5-heneicosylresorcinol peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of 5-heneicosylresorcinol in the test solution, and calculate the percentage content of 5-heneicosylresorcinol in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.010% of 5-heneicosylresorcinol ($C_{27}H_{48}O_2$), calculated with reference to the dried substance.