Asparagi Radix

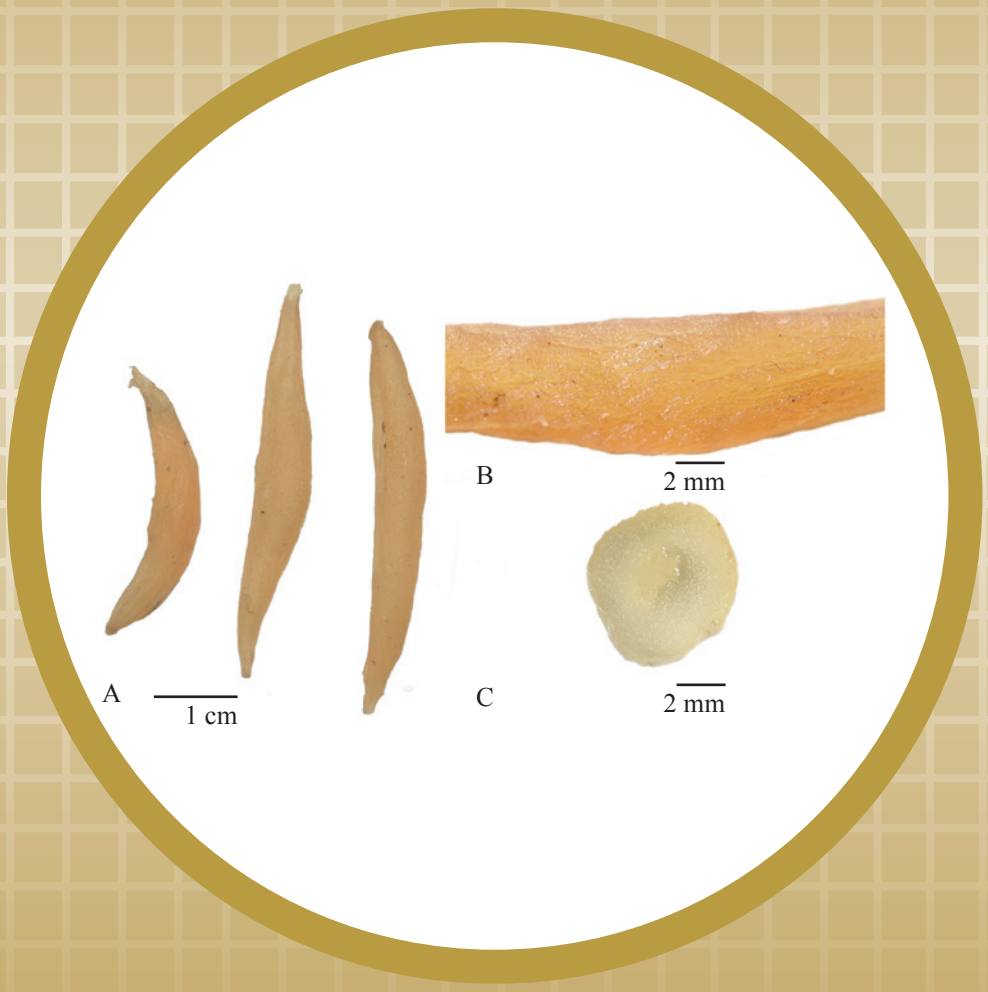

Figure 1 A photograph of Asparagi Radix

A. Asparagi Radix

B. Magnified image of root tuber

C. Magnified image of cross section

1. NAMES

Official Name: Asparagi Radix

Chinese Name: 天冬

Chinese Phonetic Name: Tiandong

2. SOURCE

Asparagi Radix is the dried root tuber of *Asparagus cochinchinensis* (Lour.) Merr. (Liliaceae). The root tuber is collected in autumn and winter, washed clean, steamed or boiled until the centre is devoid of core white; the velamen peeled while hot, washed, then bake to dryness at lower than 50°C for 20 hour or dried under the sun to obtain Asparagi Radix.

3. DESCRIPTION

Long-fusiform, slightly curved, 5-18 cm long, 5-20 mm in diameter. Externally yellowish-white to yellowish-brown, translucent, smooth or with deep and shallow longitudinal wrinkles, patches of the outer peel of greyish-brown colour occasionally found. Texture hard or tough and sleek, fracture horny, mucilaginous, stele yellowish-white. Odour slight; taste sweet and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Velamen peeled off. Cortex broad, with several mucilage cells which contain raphides of calcium oxalate. Endodermis distinct, with fibre bundles scattered beneath, stone cells occasionally found. Phloem alternately arranged with xylem strands. Xylem mostly arranged in a T shape; vessels sometimes penetrated into the pith. Pith broad, parenchymatous cells contain raphides of calcium oxalate (Fig. 2).

Powder

Colour yellowish-white. Raphides of calcium oxalate mostly in bundles or scattered singly; polychromatic under the polarized microscope. Lignified parenchymatous cells numerous, usually broken, wall slightly thickened, with distinct pits and pit canals. Fibres scattered or in bundles, walls slightly thickened, with distinct pits and pit canals; white under the polarized microscope. Bordered-pitted vessels visible, 18-109 μm in diameter. Stone cells rare; usually scattered singly, long-elliptical, 80-430 μm long, 36-88 μm in diameter, walls 10-35 μm thick, with striations, distinct pits and pit canals; white under the polarized microscope (Fig. 3).

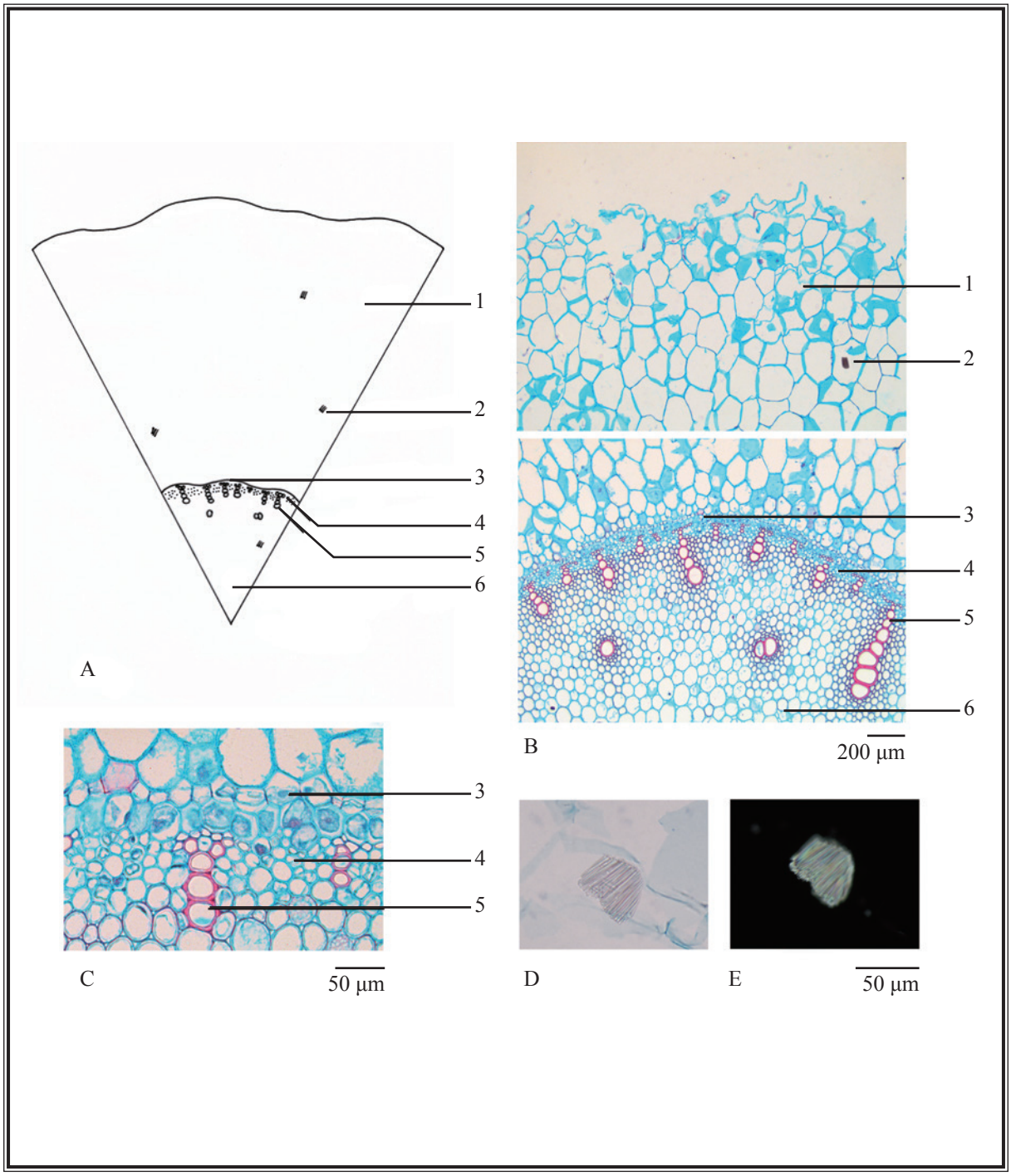

Figure 2 Microscopic features of transverse section of Asparagi Radix

A. Sketch B. Section illustration C. Section magnified
D. Raphides of calcium oxalate (under the light microscope)
E. Raphides of calcium oxalate (under the polarized microscope)

1. Cortex 2. Raphides of calcium oxalate 3. Endodermis 4. Phloem 5. Xylem 6. Pith

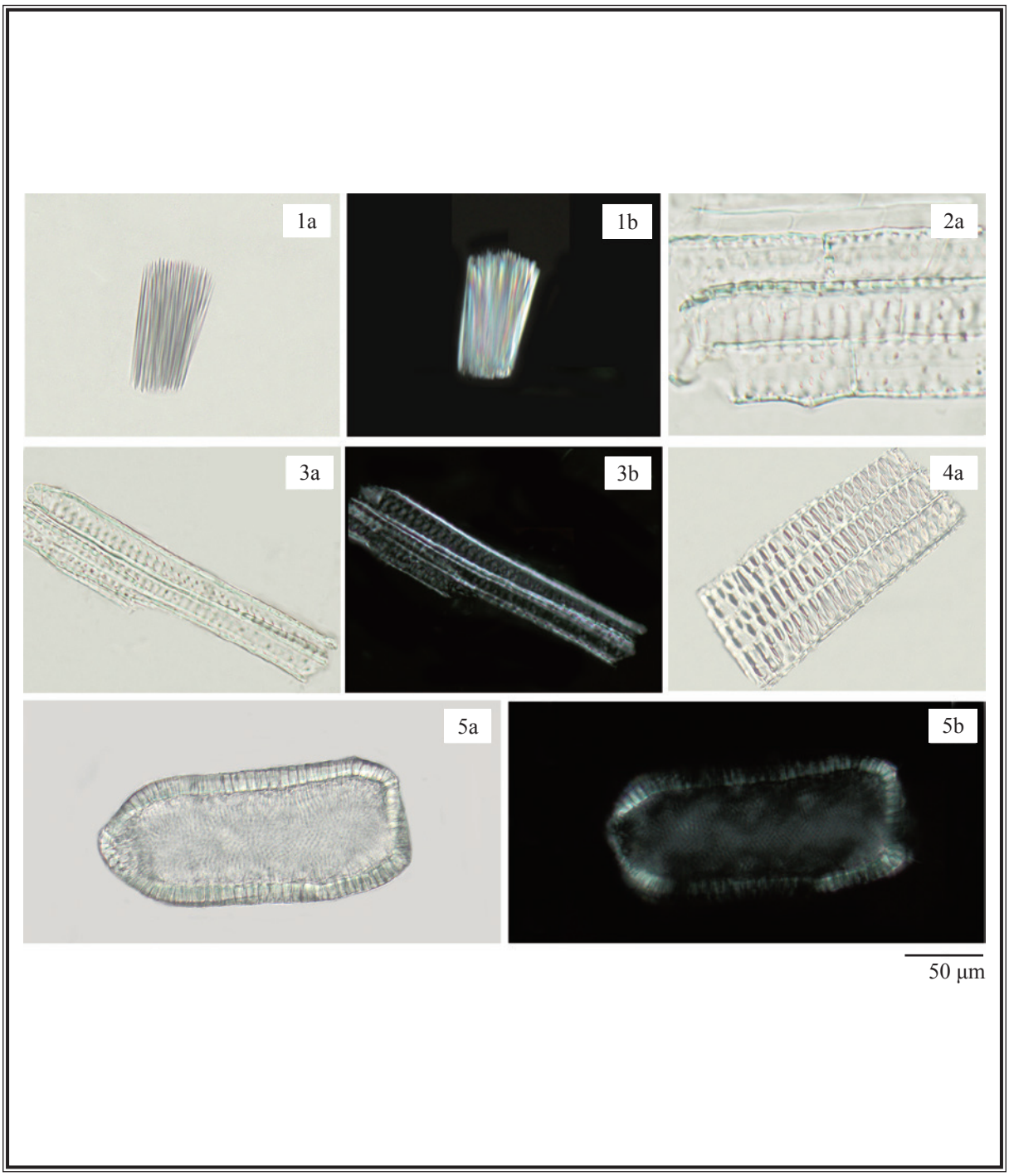

Figure 3 Microscopic features of powder of Asparagi Radix

1. Raphides of calcium oxalate 2. Lignified parenchymatous cells
3. Fibres 4. Bordered-pitted vessels 5. Stone cell

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

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

Standard solution

Pseudoprotodioscin standard solution

Weigh 1.0 mg of pseudoprotodioscin CRS (Fig. 4) and dissolve in 1 mL of methanol (65%).

Developing solvent system

Prepare a mixture of water, acetonitrile and tetrahydrofuran (6:3:1, v/v).

Spray reagent

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

Test solution

Weigh 0.5 g of the powdered sample and place it in a 10-mL centrifuge tube, then add 2.5 mL of methanol (65%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Pipette 1 mL of supernatant to 2 mL of water. Pre-condition a solid-phase extraction (SPE) column containing ODS packing (3 mL, 200 mg) with 3 mL of methanol and then followed by 3 mL of methanol (5%). Load the sample solution to the pre-conditioned SPE column. Add 2 mL of methanol (10%) to the column and discard the eluant. Add 0.5 mL of methanol to the column and collect the eluant.

Procedure

Carry out the method by using a HPTLC RP-18 F_{254S} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately pseudoprotodioscin standard solution (2 μL) and the test solution (4.5 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 min). Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

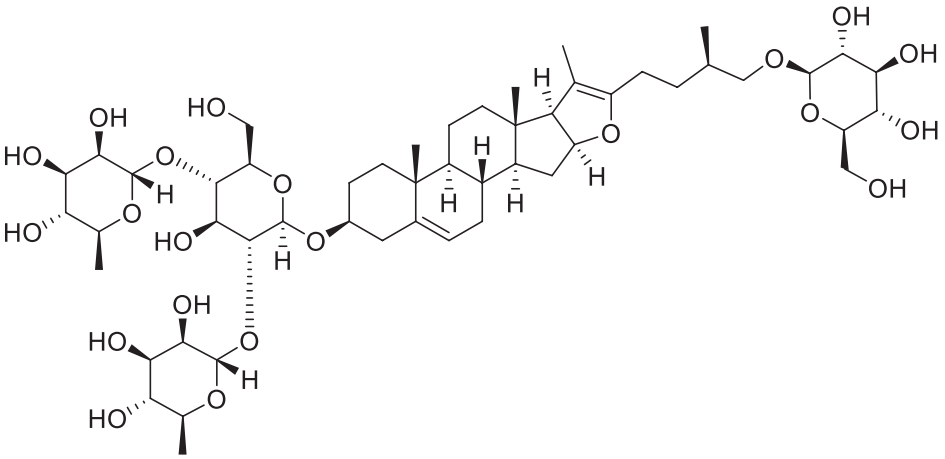

Figure 4 Chemical structure of pseudoprotodioscin

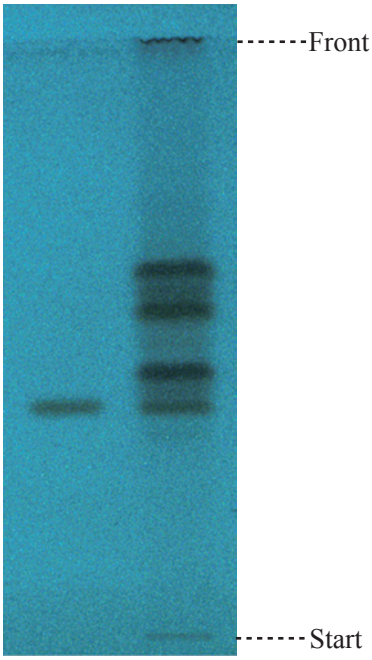

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Figure 5 A reference HPTLC chromatogram of Asparagi Radix extract observed under UV light (254 nm) after staining

1. Pseudoprotodioscin 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 pseudoprotodioscin (Fig. 5).

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

Standard solution

Pseudoprotodioscin standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of pseudoprotodioscin CRS and dissolve in 10 mL of methanol (65%).

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 (65%). Sonicate (120 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 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)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	85	15	isocratic
5 – 25	85 → 70	15 → 30	linear gradient
25 – 35	70	30	isocratic
35 – 60	70 → 69	30 → 31	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of pseudoprotodioscin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of pseudoprotodioscin should not be more than 5.0%; the RSD of the retention time of pseudoprotodioscin peak should not be more than 2.0%; the column efficiency determined from pseudoprotodioscin peak should not be less than 30000 theoretical plates.

The *R* value between peak 7 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject pseudoprotodioscin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of pseudoprotodioscin peak in the chromatogram of pseudoprotodioscin Std-FP and the retention times of the eight characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify pseudoprotodioscin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of pseudoprotodioscin Std-FP. The retention times of pseudoprotodioscin 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 eight characteristic peaks of Asparagi Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the eight characteristic peaks of Asparagi Radix extract

Peak No.	RRT	Acceptable Range
1	0.75	± 0.03
2	0.77	± 0.03
3	0.80	± 0.03
4	0.84	± 0.03
5	0.87	± 0.03
6	0.97	± 0.03
7 (marker, pseudoprotodioscin)	1.00	-
8	1.04	± 0.03

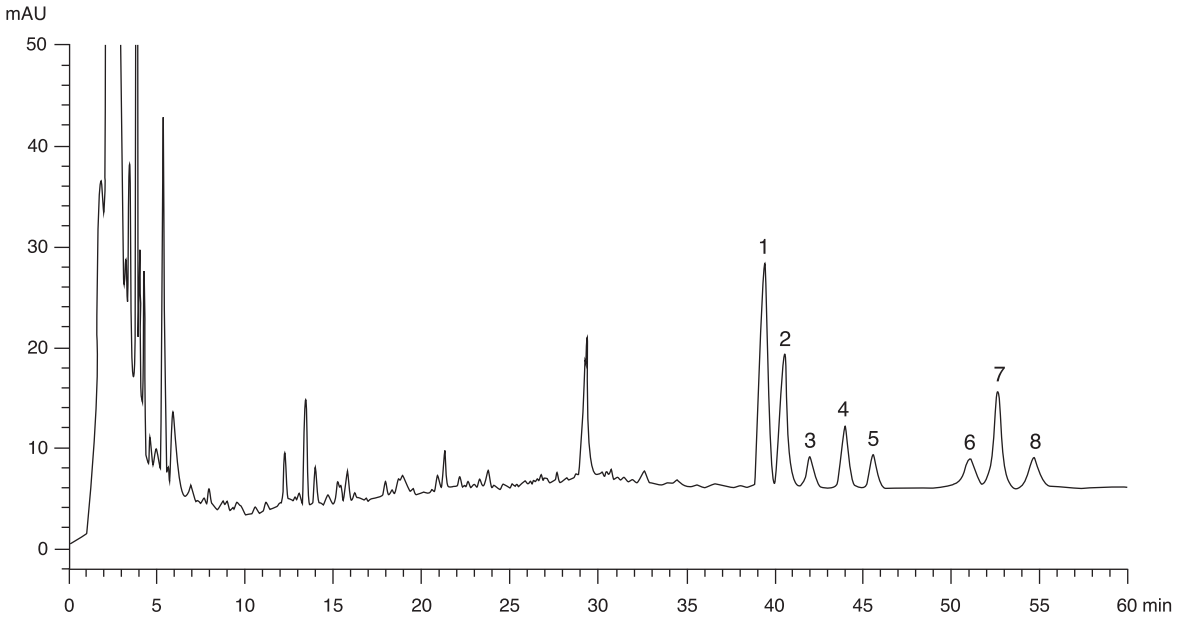

Figure 6 A reference fingerprint chromatogram of Asparagi Radix extract

For positive identification, the sample must give the above eight 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 XVI)*: not more than 400 mg/kg.

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 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 64.0%.
Ethanol-soluble extractives (cold extraction method): not less than 61.0%.

7. ASSAY

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

Standard solution

Pseudoprotodioscin standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of pseudoprotodioscin CRS and dissolve in 10 mL of methanol (65%).

Pseudoprotodioscin standard solution for assay, Std-AS

Measure accurately the volume of the pseudoprotodioscin Std-Stock, dilute with methanol (65%) to produce a series of solutions of 0.5, 5, 10, 25, 50 mg/L for pseudoprotodioscin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (65%). Sonicate (120 W) the mixture for 15 min. Centrifuge at about 4000 × g for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times with 10 mL of methanol (65%) and 5 mL of methanol (65%) respectively. Combine the supernatants and make up to the mark with methanol (65%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (203 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 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	85	15	isocratic
5 – 25	85 → 70	15 → 30	linear gradient
25 – 35	70	30	isocratic
35 – 60	70 → 69	30 → 31	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of pseudoprotodioscin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of pseudoprotodioscin should not be more than 5.0%; the RSD of the retention time of pseudoprotodioscin peak should not be more than 2.0%; the column efficiency determined from pseudoprotodioscin peak should not be less than 30000 theoretical plates.

The *R* value between pseudoprotodioscin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of pseudoprotodioscin Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of pseudoprotodioscin against the corresponding concentrations of pseudoprotodioscin 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 pseudoprotodioscin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of pseudoprotodioscin Std-AS. The retention times of pseudoprotodioscin 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 pseudoprotodioscin in the test solution, and calculate the percentage content of pseudoprotodioscin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.026% of pseudoprotodioscin ($C_{51}H_{82}O_{21}$), calculated with reference to the dried substance.

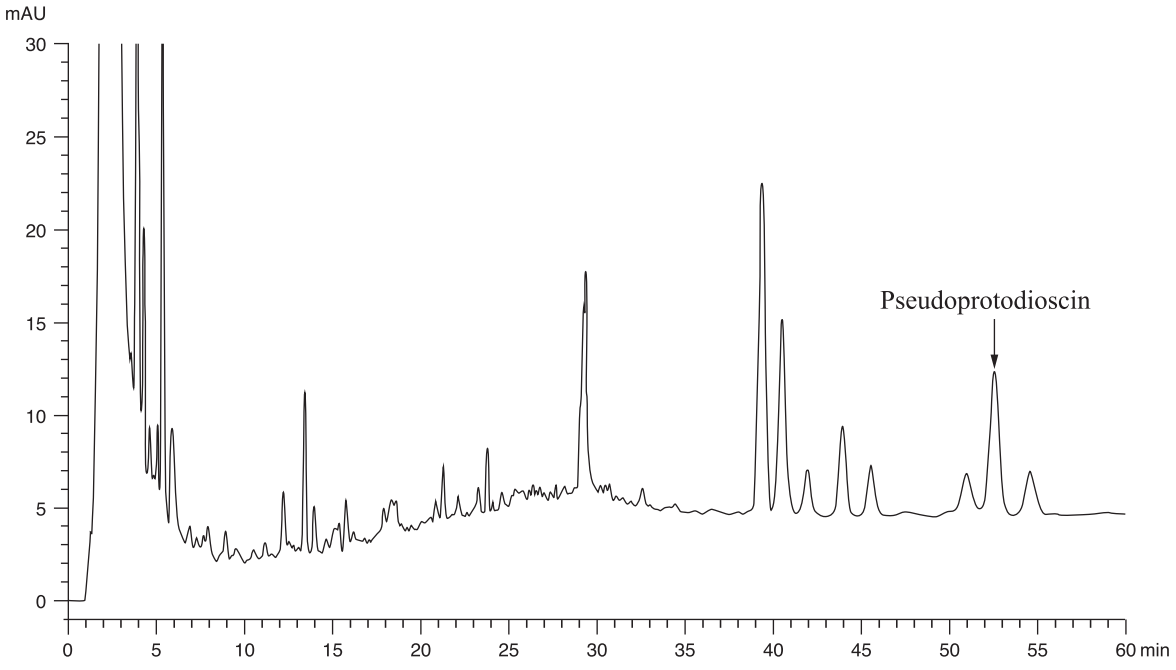

Figure 7 A reference assay chromatogram of Asparagi Radix extract