

Radix Ophiopogonis



Figure 1 A photograph of Radix Ophiopogonis

1. NAMES

Official Name: Radix Ophiopogonis

Chinese Name: 麥冬

Chinese Phonetic Name: Maidong

2. SOURCE

Radix Ophiopogonis is the dried root tuber of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. (Liliaceae). The tuber is collected in the summer, washed clean, dried under the sun and piled up until almost dry. After removal of the fibrous roots, the tuber is finally dried to obtain Radix Ophiopogonis.

3. DESCRIPTION

Spindle-shaped, both ends slightly pointed, 0.8-4.5 cm long, 2-9 mm in diameter in the middle part. Externally pale yellowish-white to yellowish-white or yellowish-brown; translucent and marked by fine, irregular and relatively deep longitudinal striae; one end often with a small exposed stele. Texture pliable; fracture yellowish-white to yellowish-brown, cuticular, translucent, with a small cylindrical stele in the centre. Odour slightly aromatic; taste sweet with a slight bitterness. Sticky when chewed (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Velamen consists of 3-5 rows of lignified cells of subsquare, subrectangular or polygonal shape. Exodermal cells wall slightly thickened. Cortex broad, scattered with mucilage cells containing raphides of calcium oxalate of 2 types: one type is thin and short, the other type is thicker and longer. Stone cell layer consisting of 1-2 rows of cells is located outside of the endodermis, the shape of the cells long polygonal to subpolygonal, with the inner and lateral wall thickened, and having dense pits. Endodermis consists of cells with evenly thickened and lignified wall, as well as passage cells. Pericycle relatively small, consisting of 1-2 rows of parenchyma cells. Phloem bundles 16-22, located between two star-angles of the xylem bundles. Xylem consists of vessels, tracheids, xylem fibres and lignified cells in the inner side, linking up to a ring. Pith small, with suborbicular parenchyma cells (Fig. 2).

Powder

Colour yellowish-white. Raphides of calcium oxalate abundant, scattered or in bundles located in suborbicular to elliptic mucilage cells; the thin type 12-65 μm long, some are thicker and longer, up to 129 μm in length. Bright orange or polychromatic observed under the polarized microscope. Stone cells usually in groups; colourless; subsquare, rectangular or polygonal, 21-57 μm in diameter; walls thickened unevenly; pit canals distinct, pits dense, flat-ellipsoid or short slit-shaped. Bright white in colour is observed under the polarized microscope. Cells of the endodermis rectangular or band-shaped; the pit canals distinct; pits dotted. Xylem fibres slender, their ends oblique or straight, the wall slightly thickened; pits oblique slit-shaped, cross-shaped or V-shaped (Fig. 3).

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

Standard solution

Ophiopogonin D standard solution

Weigh 1.0 mg of ophiopogonin D CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (8:2:0.3, v/v).

Spray reagent

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

Test solution

Weigh 5.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of methanol. Reflux the mixture for 60 min. Cool down to room temperature and filter. Wash the residue with 10 mL of methanol. Combine the filtrate and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 25 mL of water. Transfer the mixture to a 100-mL separatory funnel. Extract with 25 mL of water-saturated 1-butanol. Collect the 1-butanol extract and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of methanol and then filter.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately ophiopogonin D standard solution (2 μL) and the test solution (4 μL) 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 120°C until the spots or bands become visible (about 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

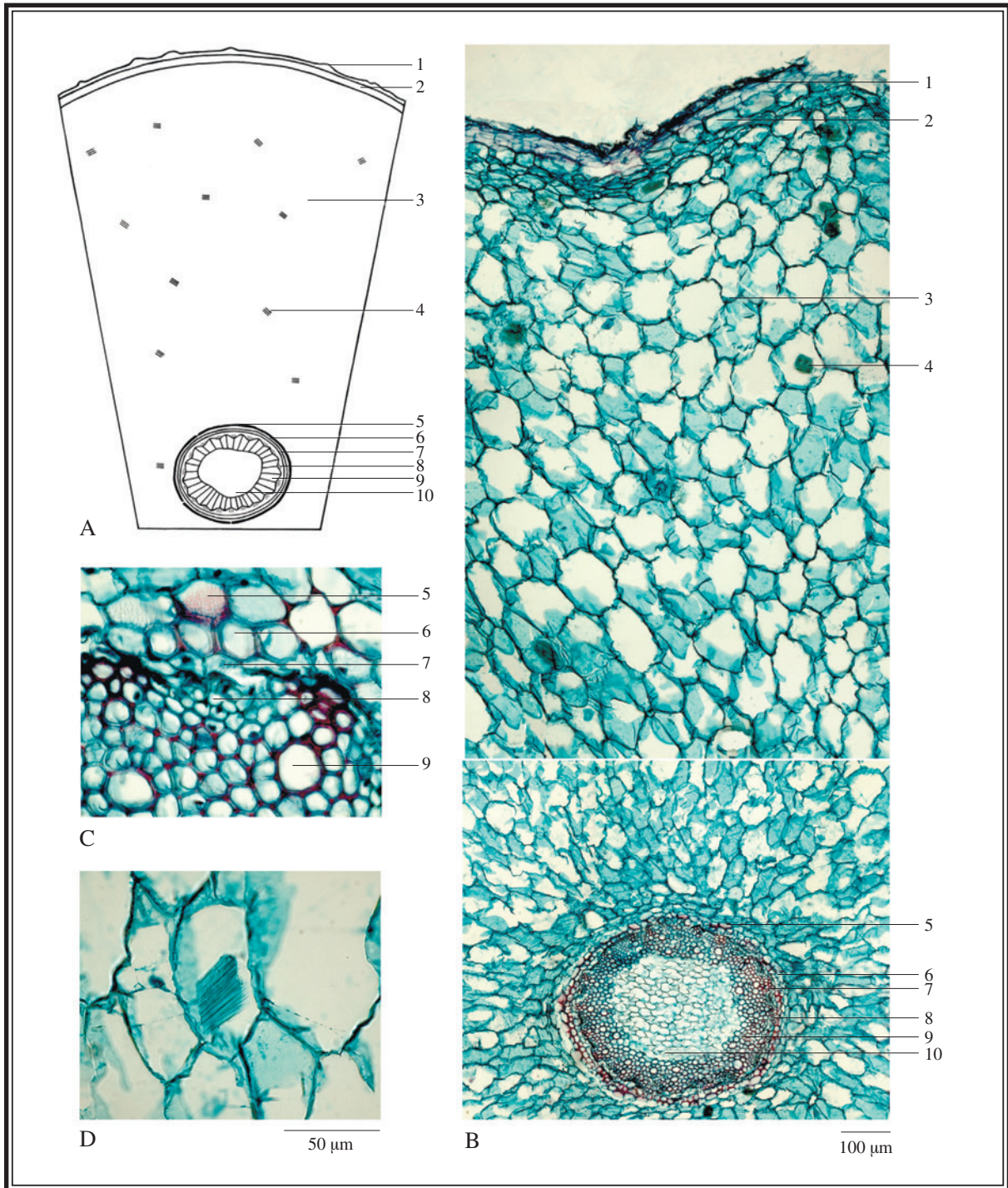


Figure 2 Microscopic features of transverse section of *Radix Ophiopogonis*

A. Sketch B. Section illustration C. Endodermis and stele

D. Raphides of calcium oxalate

1. Velamen 2. Exodermis 3. Cortex 4. Raphides of calcium oxalate

5. Stone cells layer 6. Endodermis 7. Pericycle 8. Phloem 9. Xylem 10. Pith

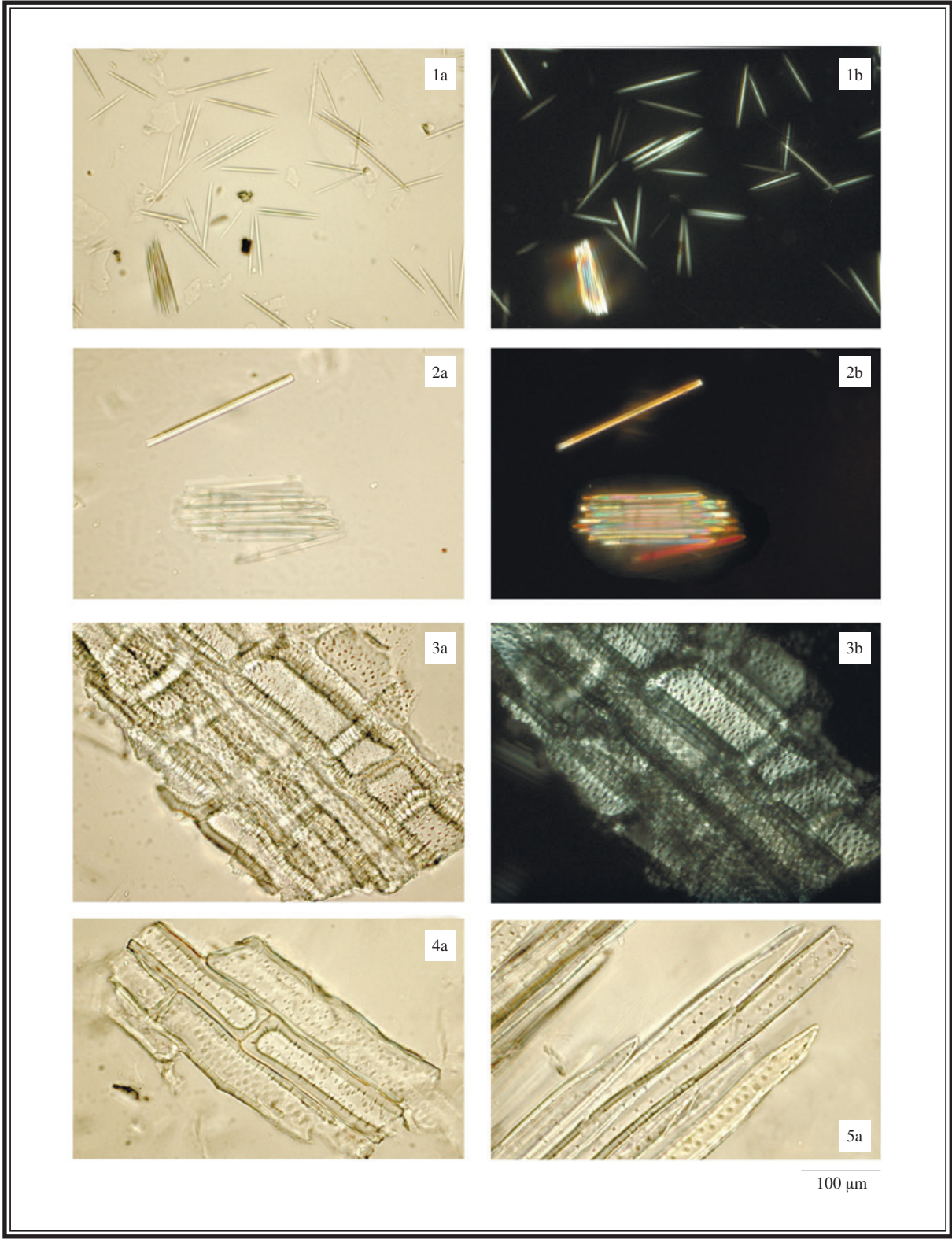


Figure 3 Microscopic features of powder of Radix Ophiopogonis

- 1. Raphides of calcium oxalate (thin)
- 2. Raphides of calcium oxalate (thick and long)
- 3. Stone cells
- 4. Endodermal cells
- 5. Xylem fibres

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

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 ophiopogonin D.

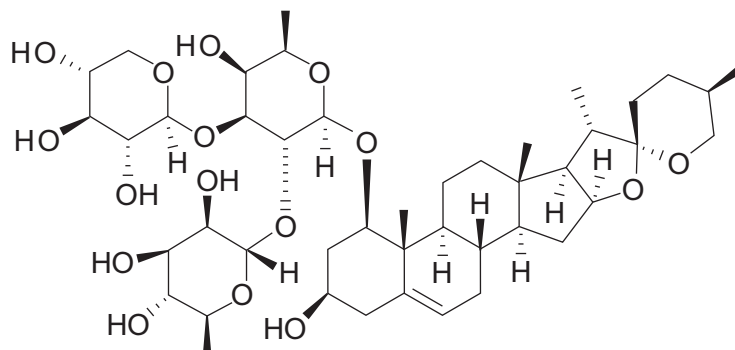


Figure 4 Chemical structure of ophiopogonin D

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Ophiopogonin D standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of ophiopogonin D CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of methanol. Reflux the mixture for 60 min. Cool down to room temperature and filter. Wash the residue with 10 mL of methanol. Combine the filtrate and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 25 mL of water. Transfer the mixture to a 100-mL separatory funnel. Extract with 25 mL of water-saturated 1-butanol. Collect the 1-butanol extract and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of methanol. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 100°C; nebulizer gas (N_2) flow: 2.0 L/min] and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The column temperature is maintained at 30°C during the separation. 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
0–20	75 \rightarrow 60	25 \rightarrow 40	linear gradient
20–50	60 \rightarrow 50	40 \rightarrow 50	linear gradient
50–60	50 \rightarrow 35	50 \rightarrow 65	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of ophiopogonin D Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ophiopogonin D should not be more than 3.0%; the RSD of the retention time of ophiopogonin D peak should not be more than 2.0%; the column efficiency determined from ophiopogonin D peak should not be less than 80000 theoretical plates.

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 ophiopogonin D Std-FP and the test solution (20 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of ophiopogonin D peak in the chromatogram of ophiopogonin D Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify ophiopogonin D peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ophiopogonin D Std-FP. The retention times of ophiopogonin D 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 Radix Ophiopogonis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Radix Ophiopogonis extract

Peak No.	RRT	Acceptable Range
1	0.51	± 0.03
2	0.64	± 0.03
3	0.91	± 0.03
4 (marker, ophiopogonin D)	1.00	-
5	1.05	± 0.03

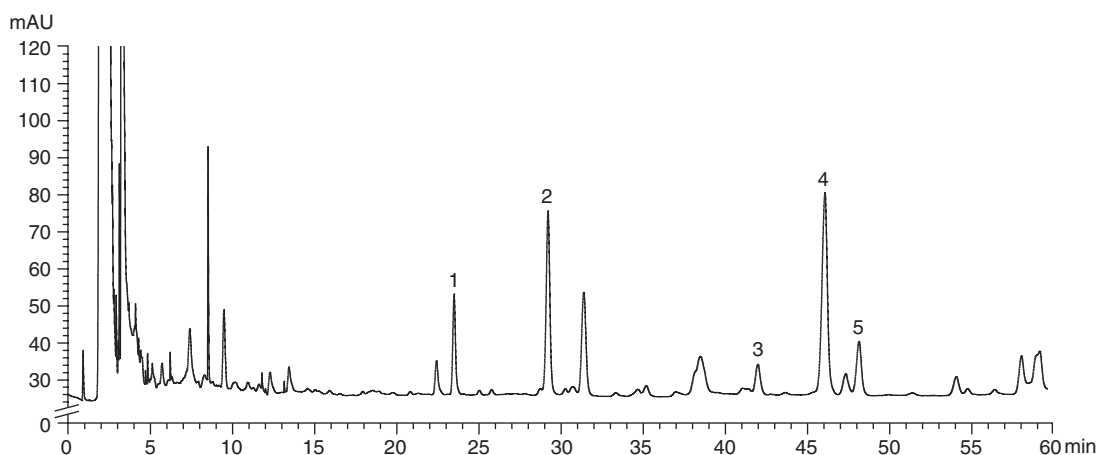


Figure 5 A reference fingerprint chromatogram of Radix Ophiopogonis extract

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 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 XV*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 2.5%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*): not more than 18.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 60.0%.

Ethanol-soluble extractives (cold extraction method): not less than 43.0%.

7. ASSAY

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

Standard solution

Ophiopogonin D standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of ophiopogonin D CRS and dissolve in 5 mL of methanol.

Ophiopogonin D standard solution for assay, Std-AS

Measure accurately the volume of the ophiopogonin D Std-Stock, dilute with methanol to produce a series of solutions of 100, 200, 300, 400, 500 mg/L for ophiopogonin D.

Test solution

Weigh accurately 3.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 100 mL of methanol. Reflux the mixture for 60 min. Cool down to room temperature and filter. Wash the residue with 10 mL of methanol. Combine the filtrate and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 25 mL of water. Transfer the mixture to a 100-mL separatory funnel. Wash the flask with 5 mL of water. Combine the solution. Extract with 30 mL of water-saturated 1-butanol. Allow to stand for overnight. Collect the 1-butanol extract and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 100°C; nebulizer gas (N₂) flow: 2.0 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The column temperature is maintained at 30°C during the separation. 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
0–40	60 → 53.5	40 → 46.5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of ophiopogonin D Std-AS (400 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ophiopogonin D should not be more than 3.0%; the RSD of the retention time of ophiopogonin D peak should not be more than 2.0%; the column efficiency determined from ophiopogonin D peak should not be less than 15000 theoretical plates.

The *R* value between ophiopogonin D peak and the closest peak in the chromatogram of the test solution should not be less than 1.4.

Calibration curve

Inject a series of ophiopogonin D Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of ophiopogonin D against the natural logarithm of the corresponding concentrations of ophiopogonin D 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 ophiopogonin D peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ophiopogonin D Std-AS. The retention times of ophiopogonin D 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 ophiopogonin D in the test solution by using the following equation –

$$\text{Concentration of ophiopogonin D in the test solution} = e^{[\ln(A)-I]/m}$$

Where

- A = the peak area of ophiopogonin D in the test solution,
- I = the y-intercept of the 5-point calibration curve of ophiopogonin D,
- m = the slope of the 5-point calibration curve of ophiopogonin D.

Calculate the percentage contents of ophiopogonin D in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.010% of ophiopogonin D (C₄₄H₇₀O₁₆), calculated with reference to the dried substance.