Omphalia

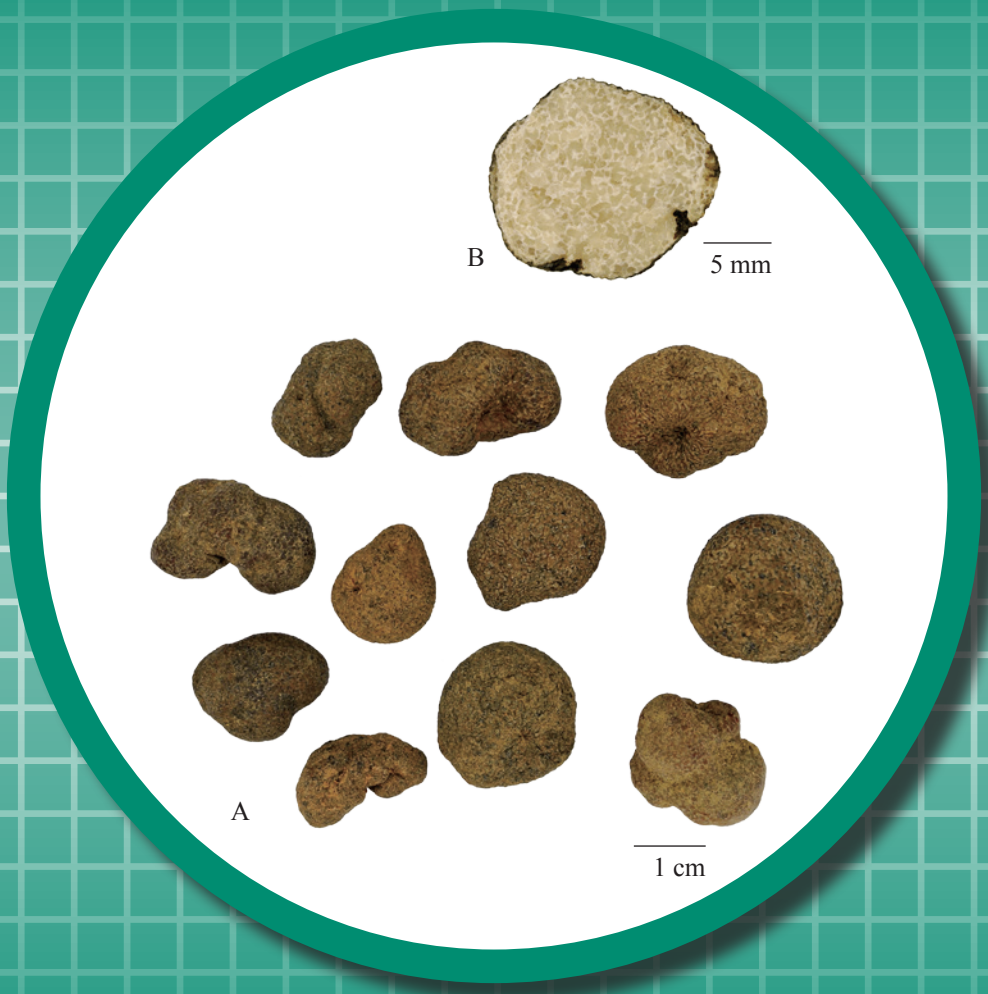

Figure 1 A photograph of Omphalia

A. Omphalia B. Magnified transverse section of sclerotium

1. NAMES

Official Name: Omphalia

Chinese Name: 雷丸

Chinese Phonetic Name: Leiwan

2. SOURCE

Omphalia is the dried sclerotium of *Omphalia lapidescens* Schroet. (Polyporaceae). The sclerotium is collected in autumn, washed clean and dried under the sun to obtain Omphalia.

3. DESCRIPTION

Subspheroid or irregular masses, 7-40 mm in diameter. Externally greyish-brown or blackish-brown, with irregular, slightly protuberant and fine reticulate striations. Texture hard and compact, uneasily broken. Fracture uneven, white or pale greyish-yellow, usually with yellowish-brown marble-like striations. Odour slight; taste slightly bitter, granular; minor tenacious sensation when chewed; no residue after prolonged chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Yellowish-brown or reddish-brown hyphae located in the outer layer, rind-like. Colourless hyphae occupied the interior part, arranged densely. Crystals of calcium oxalate scattered among the colourless hyphae, mainly with clusters of calcium oxalate, prisms of calcium oxalate and microcrystals. Cluster of calcium oxalate 10-73 μm in diameter (Fig. 2).

Powder

Colour greyish-yellow, brown or blackish-brown. Hyphae abundant, mainly colourless, few yellowish-brown or brownish-red, aggregate together to form irregular masses, varying in size. Scattered hyphae short, with branches, 2-6 μm in diameter. Crystals of calcium oxalate frequently occur, mainly clusters of calcium oxalate, prisms of calcium oxalate and microcrystals; polychromatic under the polarized microscope. Abundant raphides of calcium sulphate observed after adding 20% sulphuric acid; bright white under the polarized microscope (Fig. 3).

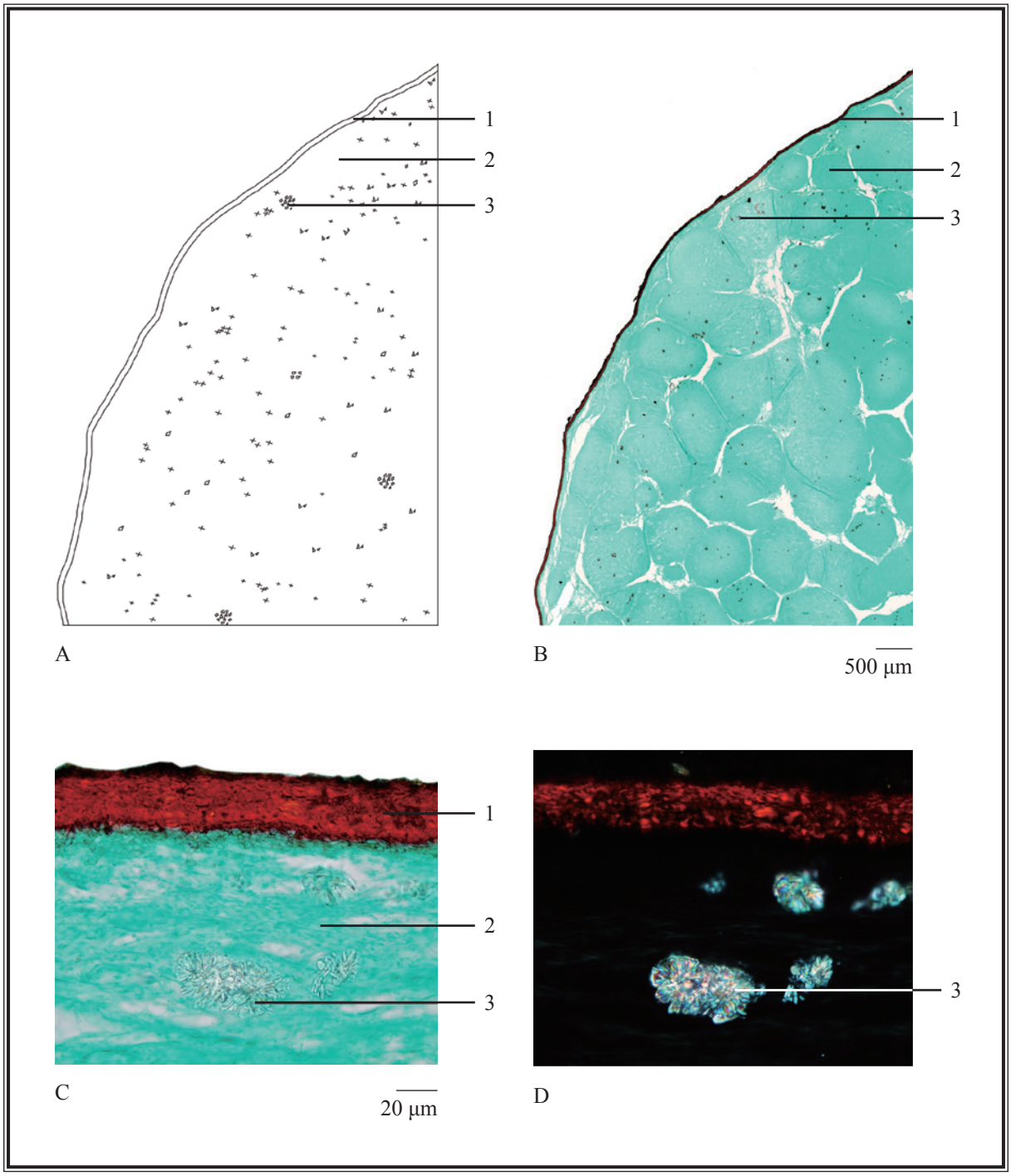

Figure 2 Microscopic features of transverse section of Omphalia

A. Sketch B. Section illustration C. Section magnified
D. Section magnified (under the polarized microscope)

1. Reddish-brown hyphae 2. Colourless hyphae 3. Crystal of calcium oxalate

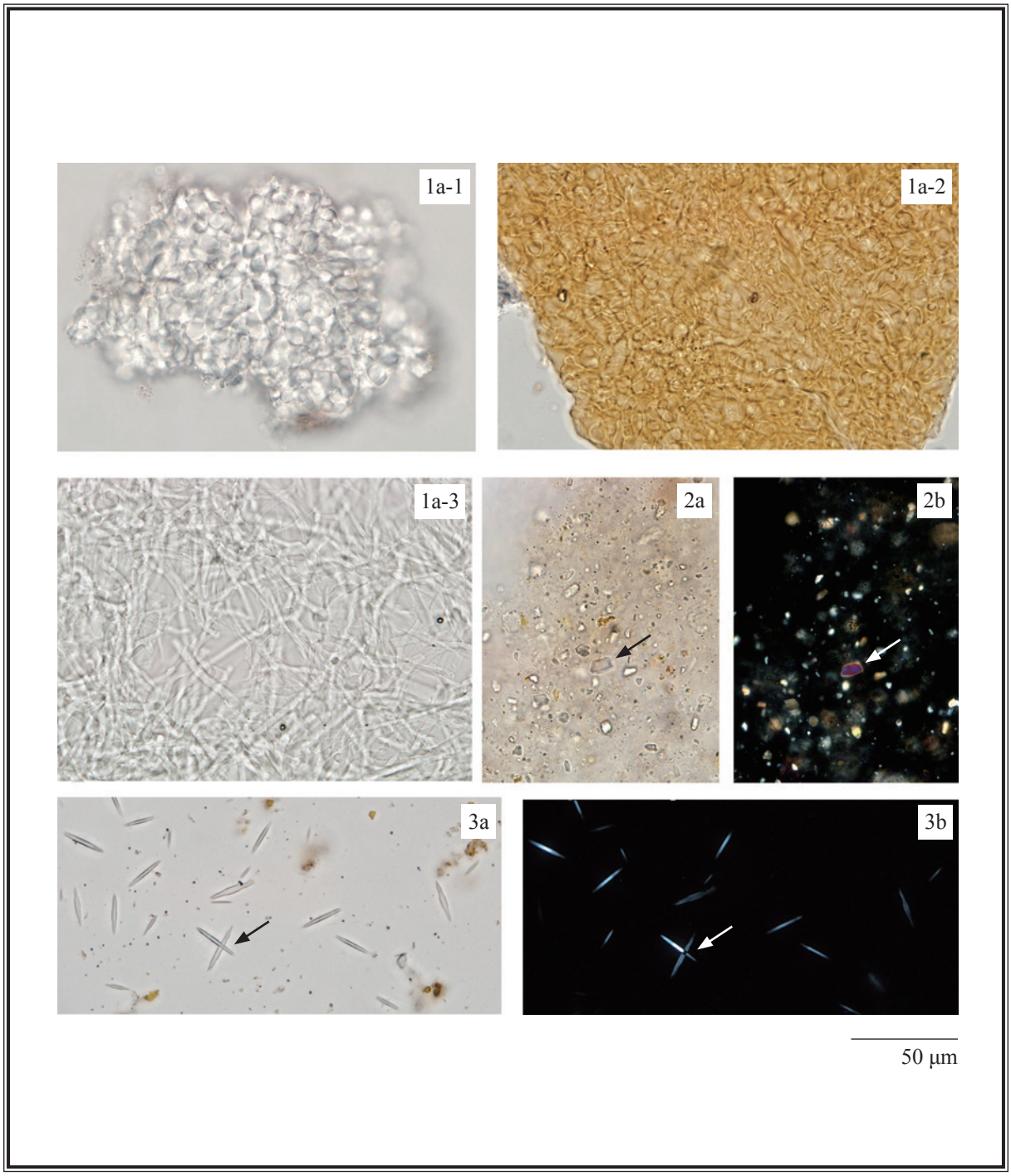

Figure 3 Microscopic features of powder of Omphalia

1. Hyphae (1-1 aggregated colourless hyphae, 1-2 aggregated yellowish-brown hyphae, 1-3 scattered colourless hyphae) 2. Crystals of calcium oxalate 3. Raphides of calcium sulphate (after adding 20% sulphuric acid)

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

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

Standard solution

Ergosterol standard solution

Weigh 5.0 mg of ergosterol CRS (Fig. 4) and place it in a 5-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC) and ethyl acetate (3:1, v/v).

Spray reagent

Weigh 2 g of phosphomolybdic acid hydrate and dissolve in 20 mL of ethanol.

Test solution

Weigh 6.0 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 30 mL of ethanol. Sonicate (220 W) the mixture for 30 min. 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 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately ergosterol standard solution (4 µL) and the test solution (10 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 140ºC until the spots or bands become visible (about 2 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

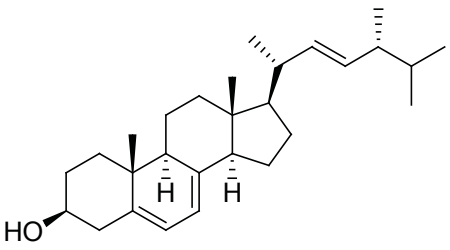

Figure 4 Chemical structure of ergosterol

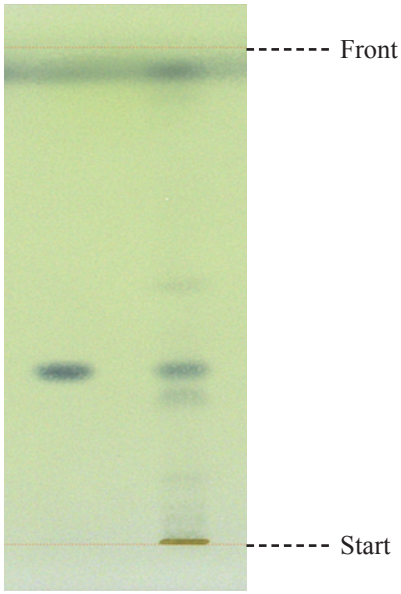

Figure 5 A reference HPTLC chromatogram of Omphalia extract observed under visible light after staining

1. Ergosterol standard solution 2. Test solution

For positive identification, the sample must give spot or band with chromatographic charactericstics, including the colour and the R_f value, corresponding to that of ergosterol (Fig. 5).

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

Standard solution

Ergosterol standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of ergosterol CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Test solution

Weigh 5.0 g of the freshly powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue for two times each with 2 mL of methanol. Transfer the solution to a 5-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 (275 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 50	65 → 0	35 → 100	linear gradient
50 – 70	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of ergosterol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ergosterol should not be more than 5.0%; the RSD of the retention time of ergosterol peak should not be more than 2.0%; the column efficiency determined from ergosterol peak should not be less than 100000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Omphalia extract

Peak No.	RRT	Acceptable Range
1	0.20	± 0.03
2	0.94	± 0.03
3	0.96	± 0.03
4 (marker, ergosterol)	1.00	-

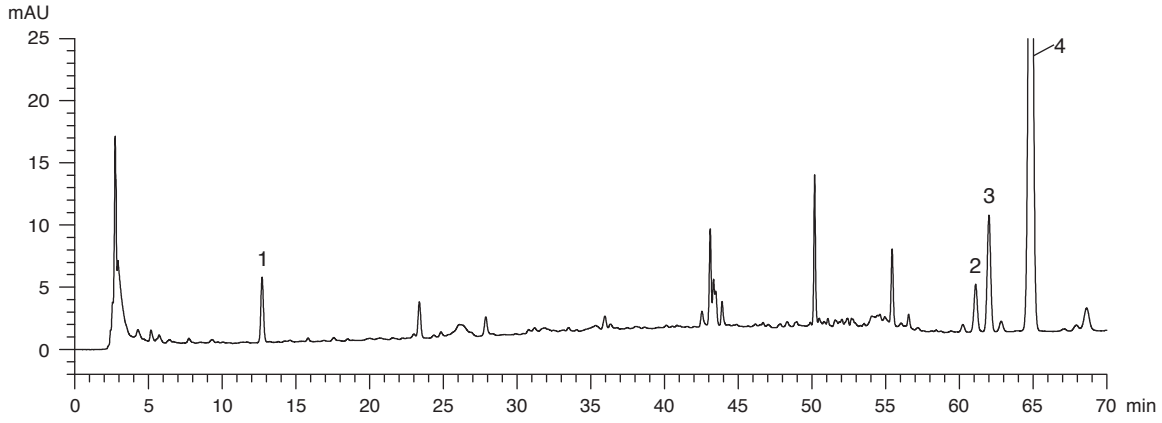

Figure 6 A reference fingerprint chromatogram of Omphalia extract

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. 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 3.0%.
Acid-insoluble ash: not more than 1.0%.

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 2.0%.
Ethanol-soluble extractives (hot extraction method): not less than 0.7%.

7. ASSAY

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

Standard solution

Ergosterol standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of ergosterol CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with methanol. Freshly prepare the standard solution.

Ergosterol standard solution for assay, Std-AS

Measure accurately the volume of the ergosterol Std-Stock, dilute with methanol to produce a series of solutions of 1, 3, 5, 7, 10 mg/L for ergosterol.

Test solution

Weigh accurately 2.0 g of the freshly powdered sample and place it in a 250-mL round-bottomed flask, then add 90 mL of methanol. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for two times each with 5 mL of methanol. Combine the solutions 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 (283 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 methanol (100%). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of ergosterol Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of ergosterol should not be more than 5.0%; the RSD of the retention time of ergosterol peak should not be more than 2.0%; the column efficiency determined from ergosterol peak should not be less than 8000 theoretical plates.

The *R* value between ergosterol 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 ergosterol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of ergosterol against the corresponding concentrations of ergosterol 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 ergosterol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ergosterol Std-AS. The retention times of ergosterol 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 ergosterol in the test solution, and calculate the percentage content of ergosterol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.020% of ergosterol ($C_{28}H_{44}O$), calculated with reference to the dried substance.