Polyporus

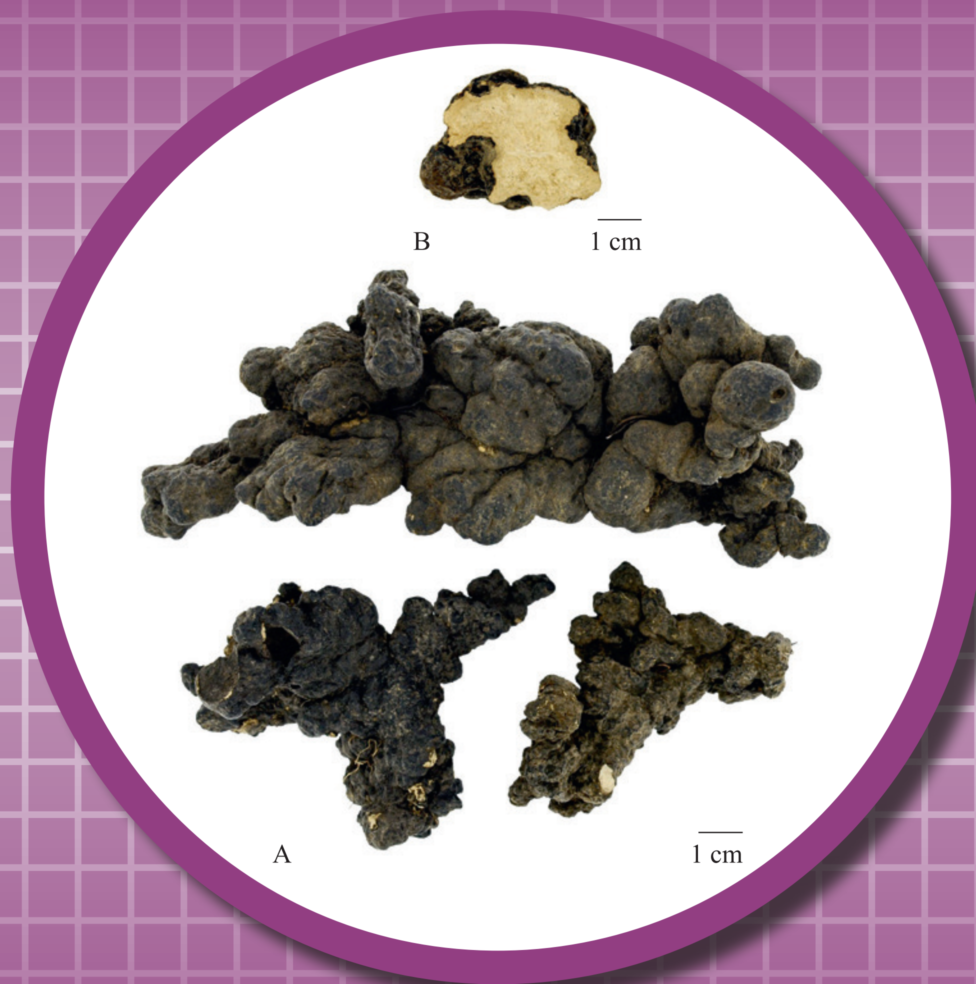

Figure 1 A photograph of Polyporus

A. Polyporus B. Fracture of sclerotium

1. NAMES

Official Name: Polyporus

Chinese Name: 豬苓

Chinese Phonetic Name: Zhuling

2. SOURCE

Polyporus is the dried sclerotium of *Polyporus umbellatus* (Pers.) Fries (Polyporaceae). The sclerotium is collected in spring and autumn, soil and foreign matter removed, then dried to obtain Polyporus.

3. DESCRIPTION

Rod-shaped, subspheroid or flat in shape, sometimes branched, 3.5-15 cm long, 7-100 mm in diameter. Externally black, greyish-black or brownish-black, crumpled or warty. Texture hard and light in weight. Fracture whitish to yellowish-white, slightly granular. Odour slight; taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Yellowish-brown to brown hyphae located in the outer layer, rind-like. Colourless hyphae occupied the interior part, arranged densely. Crystals of calcium oxalate numerous, scattered among the colourless hyphae, mainly prisms of calcium oxalate, in the form of regular octahedron, regular biconical octahedron or irregular polygonal; crystals occasionally aggregated (Fig. 2).

Powder

Colour greyish-yellow or blackish-brown. Hyphae abundant, mainly colourless, few yellowish-brown to brown hyphae aggregated to form irregular masses, varying in size; scattered hyphae sinuous, with branches or tubercular swellings, 1-14 µm in diameter. Crystals of calcium oxalate numerous, mainly prisms of calcium oxalate, in the form of regular octahedron, regular biconical octahedron or irregular polygonal, 6-54 µm in diameter; sometimes several crystals aggregated; polychromatic or white under the polarized microscope (Fig. 3).

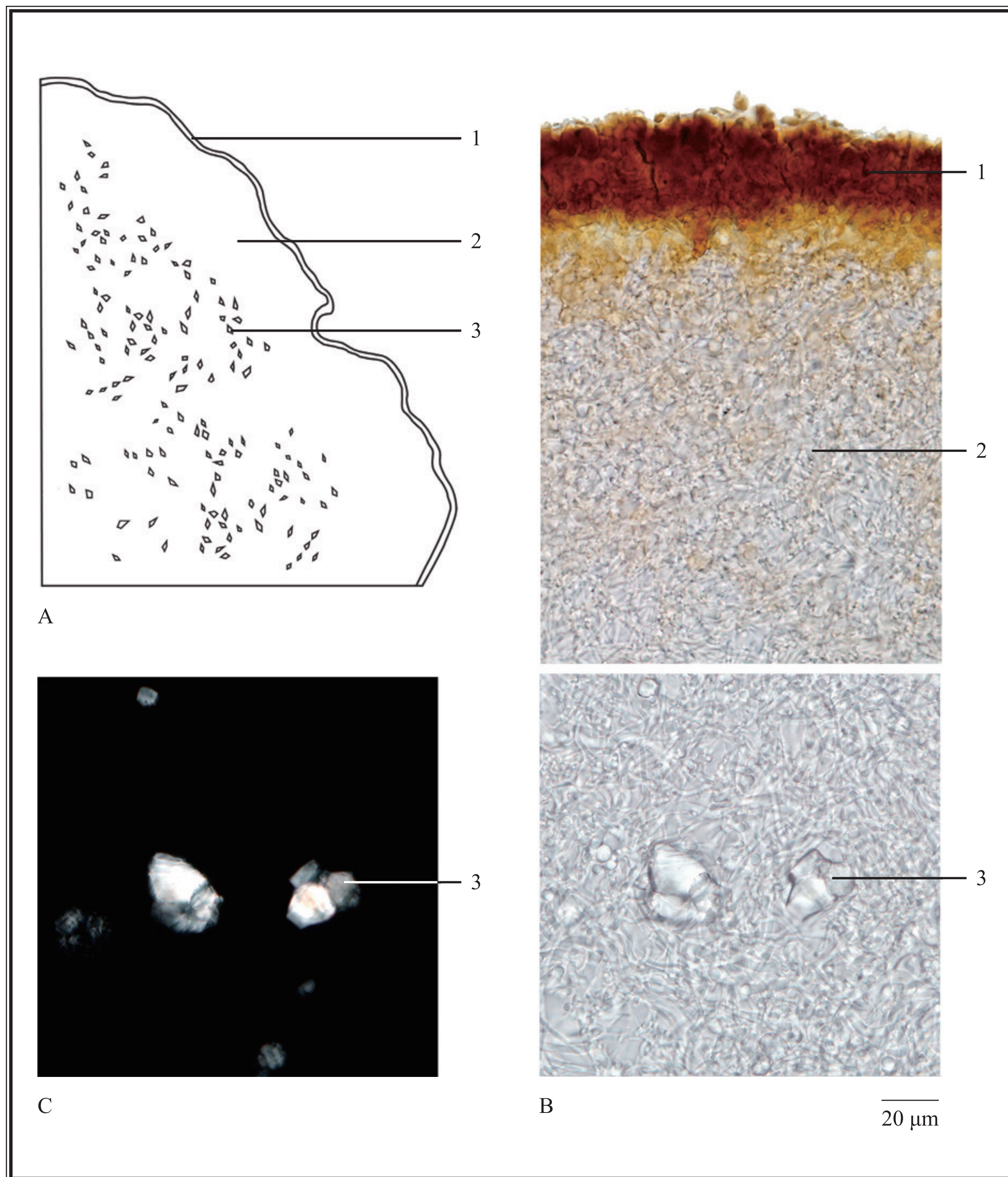

Figure 2 Microscopic features of transverse section of Polyporus

A. Sketch B. Section illustration
C. Prisms of calcium oxalate (under the polarized microscope)

1. Yellowish-brown hyphae 2. Colourless hyphae 3. Prisms of calcium oxalate

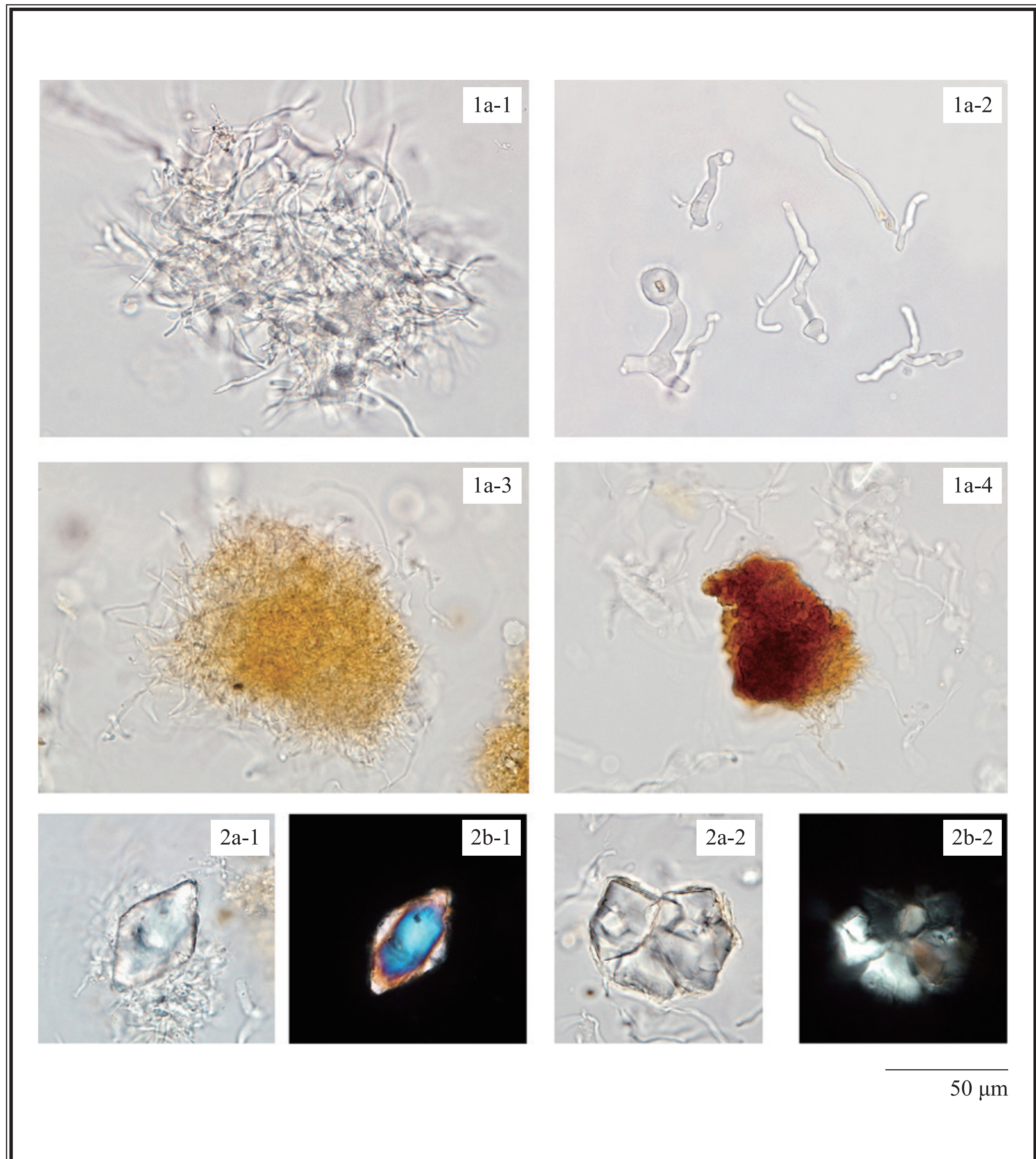

Figure 3 Microscopic features of powder of Polyporus

1. Hyphae (1-1 aggregated colourless hyphae, 1-2 scattered colourless hyphae, 1-3 aggregated yellowish-brown hyphae, 1-4 aggregated brown hyphae)
2. Prisms of calcium oxalate (2-1 scattered, 2-2 aggregated)

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 *n*-hexane and ethyl acetate (5:3, v/v).

Spray reagent

Weigh 0.5 g of phosphomolybdic acid hydrate and dissolve in 10 mL of ethanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 100-mL conical flask, then add 50 mL of methanol. Sonicate (220 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 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 and the test solution (2 μL each) 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).

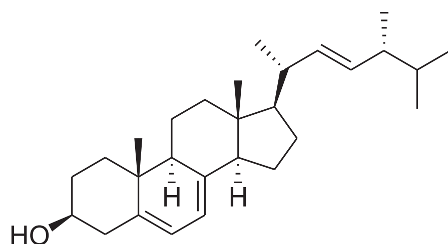

Figure 4 Chemical structure of ergosterol

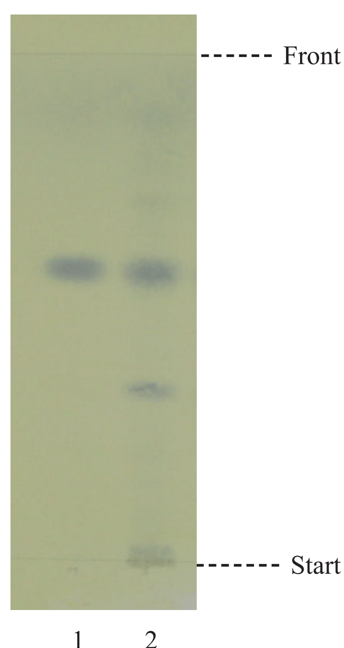

Figure 5 A reference HPTLC chromatogram of Polyporus extract observed under visible light after staining

1. Ergosterol standard solution 2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, 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 powdered sample and place it in a 100-mL conical flask, then add 50 mL of methanol. Sonicate (220 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 solutions 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 (254 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)	Methanol (%, v/v)	Water (%, v/v)	Elution
0 – 40	55 → 100	45 → 0	linear gradient
40 – 70	100	0	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 80000 theoretical plates.

The *R* value between peak 3 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 Polyporus extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.22	± 0.03
2	0.96	± 0.03
3 (marker, ergosterol)	1.00	-
4	1.06	± 0.03

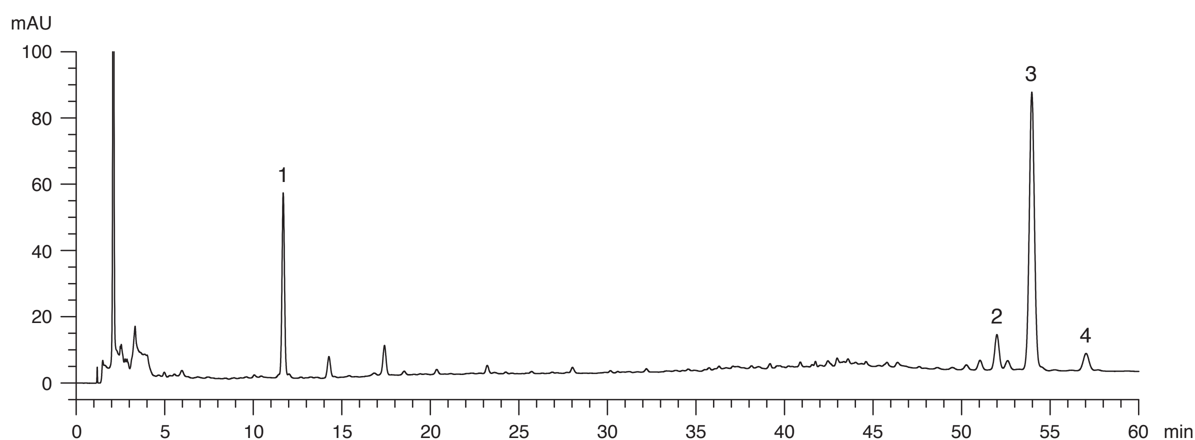

Figure 6 A reference fingerprint chromatogram of Polyporus extract

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 10.5%.
Acid-insoluble ash: not more than 4.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

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

Ergosterol standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 0.5 mg of ergosterol CRS and place it in a 5-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 2, 10, 20, 30, 40 mg/L for ergosterol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (180 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for three more times. Combine the filtrates 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 (20 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 10000 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 0.070% of ergosterol ($C_{28}H_{44}O$), calculated with reference to the dried substance.