

Rhizoma Atractylodis Macrocephalae

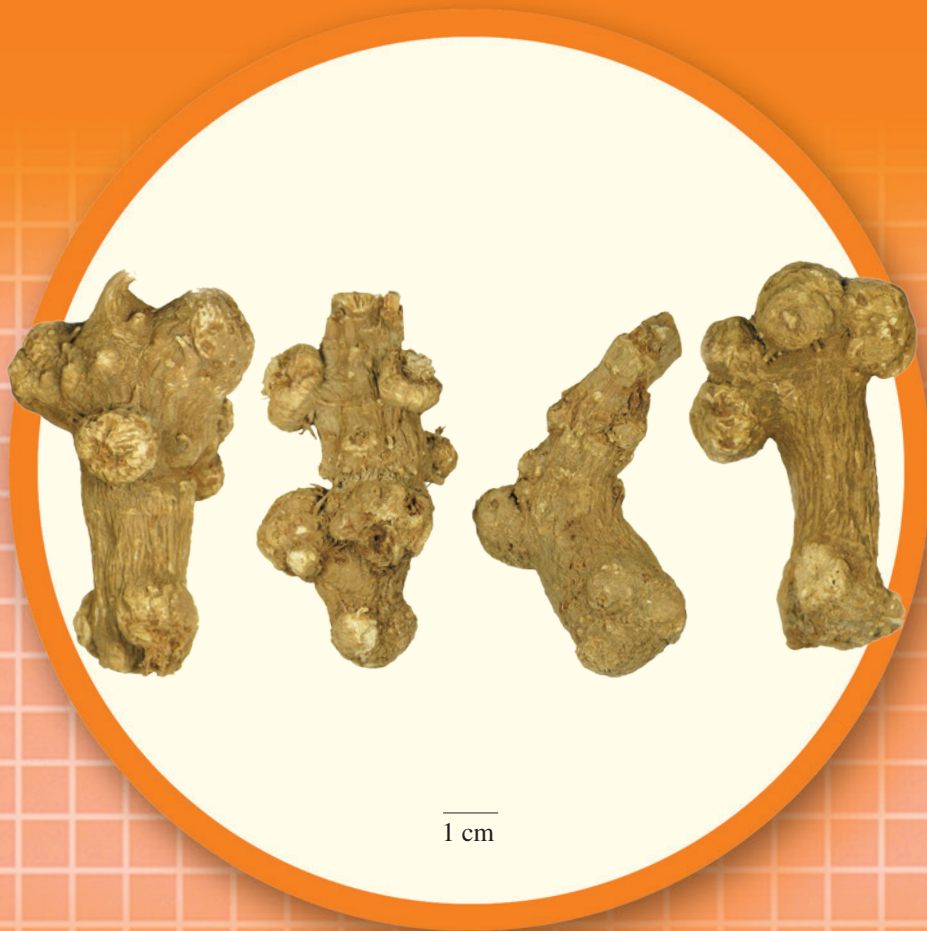


Figure 1 A photograph of Rhizoma Atractylodis Macrocephalae

Rhizoma Atractylodis Macrocephalae

1. NAMES

Official Name: Rhizoma Atractylodis Macrocephalae

Chinese Name: 白朮

Chinese Phonetic Name: Baizhu

2. SOURCE

Rhizoma Atractylodis Macrocephalae is the dried rhizome of *Atractylodes macrocephala* Koidz. (Asteraceae). The rhizome is collected in winter, when the leaves on the lower part of the plant turn yellow and those on the upper part become fragile, soil removed and baked over low heat or dried under the sun, the fibrous roots are removed to obtain Rhizoma Atractylodis Macrocephalae.

3. DESCRIPTION

Stout masses of irregular shape and size, 3-12 cm in length, 10-70 mm in diameter. Externally greyish-yellow to greyish-brown, with warty protuberances, discontinuous longitudinal wrinkles and grooves, and scars of rootlets; the top end is marked by stem remnants and bud scars. Texture hard, uneasily broken; fracture uneven, scattered with brownish-yellow, dotted oil cavities. Odour fresh and fragrant; taste sweetish and slightly pungent, viscous on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Cork consists of several rows of polygonal cells; stone cells present in groups or separately in the inner part of the cork, arranged in a discontinuous ring. Oil cavities, round to elongated-rounded in shape, containing yellow oil, numerous and scattered in the cortex, phloem and xylem. Cambium ring distinct. Vessels arranged radially, lignified, those in the internal part of the xylem surrounded by fibre bundles (Fig. 2).

Powder

Colour pale yellowish-brown. Raphides of calcium oxalate 10-36 μm in length, irregularly scattered or found in bundles in the parenchyma cells. Inulins numerous, scattered inside or outside parenchyma cells, with radial striations. Fibre cells yellow, mostly in bundles, long-fusiform, 10-62 μm in diameter, the walls fairly thickened, lignified, showing distinct

pit canals. Stone cells subglobose to polygonal or subcubical, 21-100 μm in diameter, with distinct pit canals. Vessels, reticulated and bordered-pitted, about 10-66 μm in diameter. Cork cells, polygonal, sometimes with yellowish contents (Fig. 3).

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

Standard solution

Atractylenolide III standard solution

Weigh 1.0 mg of atractylenolide III CRS (Fig. 4) and dissolve in 20 mL of ethanol.

Developing solvent system

Prepare a mixture of cyclohexane and isopropanol (8:1, v/v).

Spray reagent

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

Test solution

Weigh 2.5 g of the powdered sample and place it in a 100-mL conical flask, then add 30 mL of ethanol. Sonicate (90 W) the mixture for 30 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of ethanol. Filter through a 0.45- μm PTFE filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately atractylenolide III standard solution (5 μL) and the test solution (1 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 10 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 105°C until the spots or bands become visible (about 2 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 atractylenolide III.

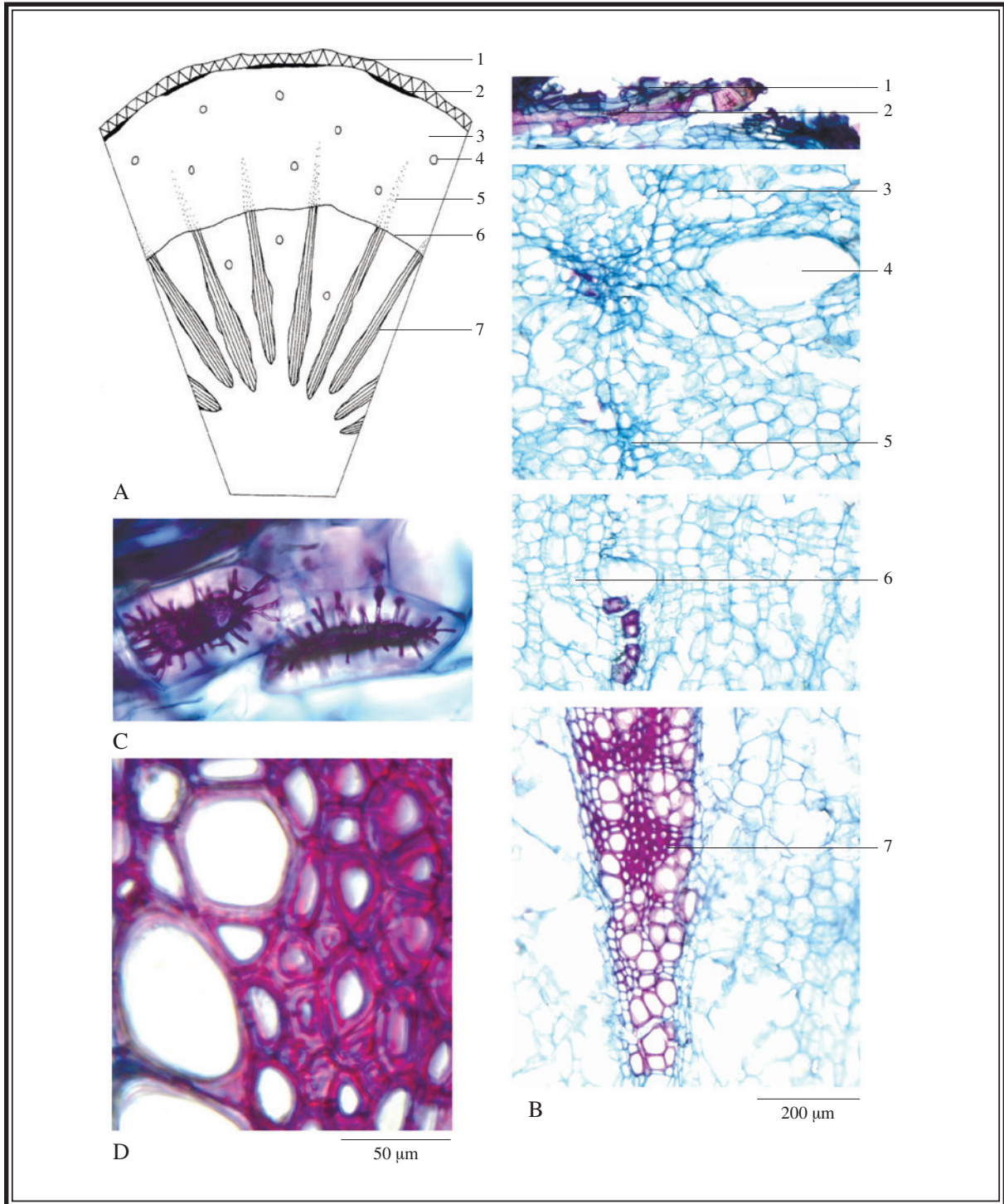


Figure 2 Microscopic features of transverse section of *Rhizoma Atractylodis Macrocephalae*

A. Sketch B. Section illustration C. Stone cells D. Xylem

1. Cork 2. Groups of stone cells 3. Cortex 4. Oil cavity 5. Phloem 6. Cambium 7. Xylem

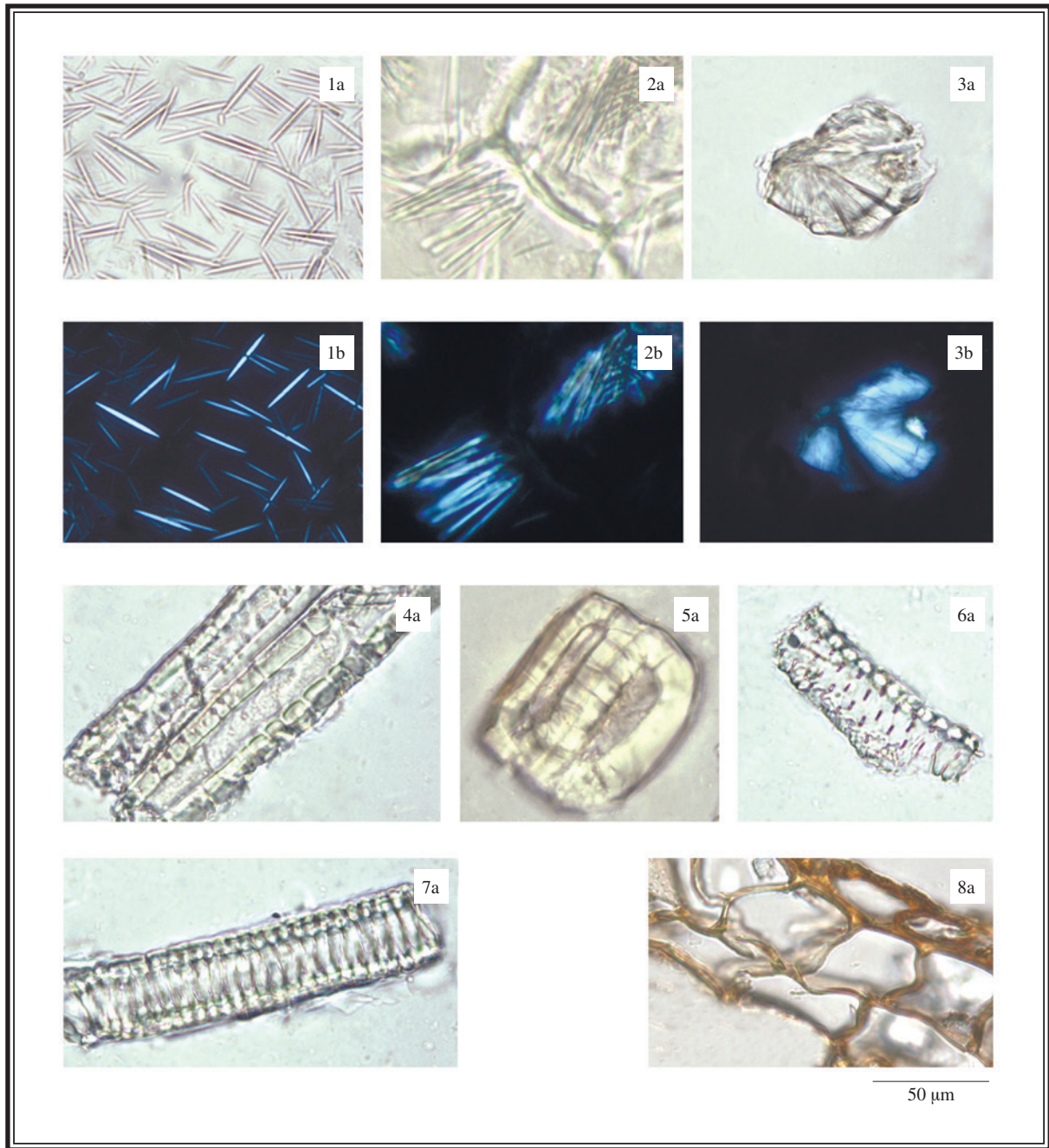


Figure 3 Microscopic features of powder of Rhizoma Atractylodis Macrocephalae

1. Irregularly scattered raphides of calcium oxalate
 2. Raphides of calcium oxalate in bundles
 3. Inulin
 4. Fibres
 5. Stone cells
 6. Bordered-pitted vessel
 7. Reticulate vessel
 8. Cork cells
- a. Features under the light microscope b. Features under the polarized microscope

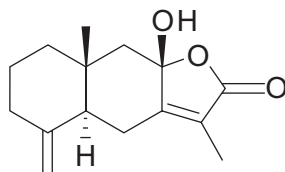


Figure 4 Chemical structure of atractylenolide III

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

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

Weigh 1.0 mg of atractylenolide III CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol. Sonicate (90 W) the mixture for 30 min. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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	80	20	isocratic
5–10	80 \rightarrow 40	20 \rightarrow 60	linear gradient
10–25	40 \rightarrow 0	60 \rightarrow 100	linear gradient
25–40	0	100	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of atractylenolide III Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of atractylenolide III should not be more than 5.0%; the RSD of the retention time of atractylenolide III peak should not be more than 2.0%; the column efficiency determined from atractylenolide III peak should not be less than 80000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject atractylenolide III Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of atractylenolide III peak in the chromatogram of atractylenolide III Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify atractylenolide III peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of atractylenolide III Std-FP. The retention times of atractylenolide III 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 Rhizoma Atractylodis Macrocephalae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Rhizoma Atractylodis Macrocephalae extract

Peak No.	RRT	Acceptable Range
1 (marker, atractylenolide III)	1.00	-
2	1.13	±0.03
3	1.16	±0.03
4 (atractylone)	1.63	±0.03
5	1.84	±0.03

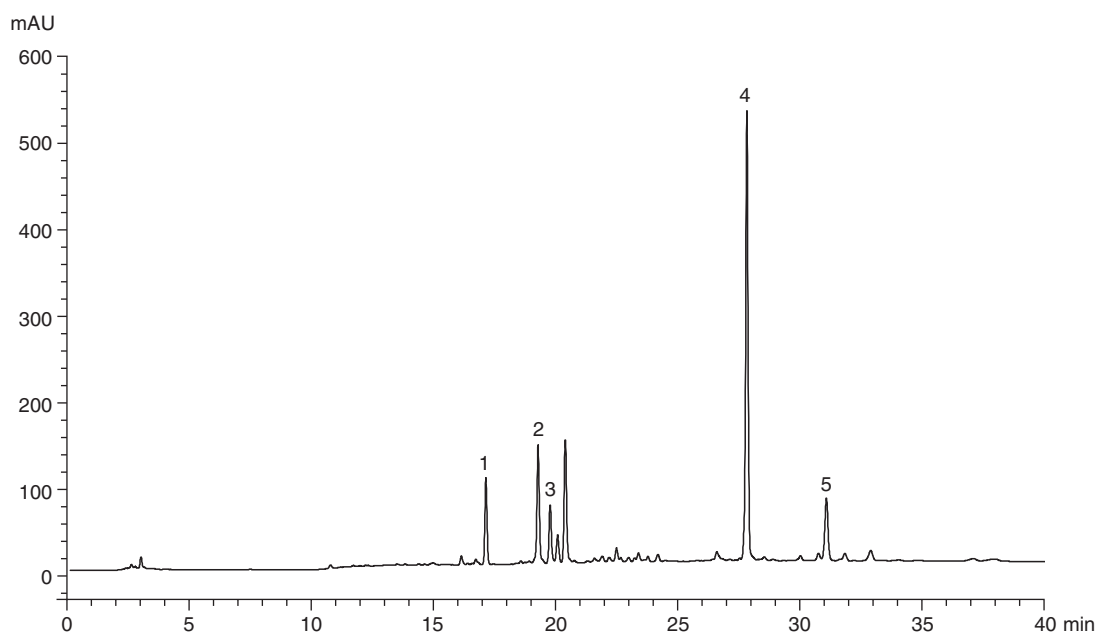


Figure 5 A reference fingerprint chromatogram of Rhizoma Atractylodis Macrocephalae extract

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*): not more than 400 mg/kg.
- 5.5 Foreign Matter** (*Appendix VIII*): not more than 1.0%.
- 5.6 Ash** (*Appendix IX*)
- Total ash: not more than 5.5%.
- Acid-insoluble ash: not more than 0.5%.
- 5.7 Water Content** (*Appendix X*): not more than 14.0%.

6. EXTRACTIVES (*Appendix XI*)

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

Ethanol-soluble extractives (hot extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

Atractylenolide III standard stock solution, Std-Stock (320 mg/L)

Weigh accurately 8.0 mg of atractylenolide III CRS and dissolve in 25 mL of methanol.

Atractylenolide III standard solution for assay, Std-AS

Measure accurately the volume of the atractylenolide III Std-Stock, dilute with methanol to produce a series of solutions of 0.2, 2.0, 16.0, 32.0, 64.0 mg/L for atractylenolide III.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the extracts and make up to the mark with ethanol (70%). Mix and filter through a 0.45- μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 nm) and a column (4.6 \times 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 (70:30, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of atractylenolide III Std-AS (32.0 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of atractylenolide III should not be more than 5.0%; the RSD of the retention time of atractylenolide III peak should not be more than 2.0%; the column efficiency determined from atractylenolide III peak should not be less than 10000 theoretical plates.

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

Limits

The sample contains not less than 0.019% of atractylenolide III ($\text{C}_{15}\text{H}_{20}\text{O}_3$), calculated with reference to the dried substance.