Acori Tatarinowii Rhizoma

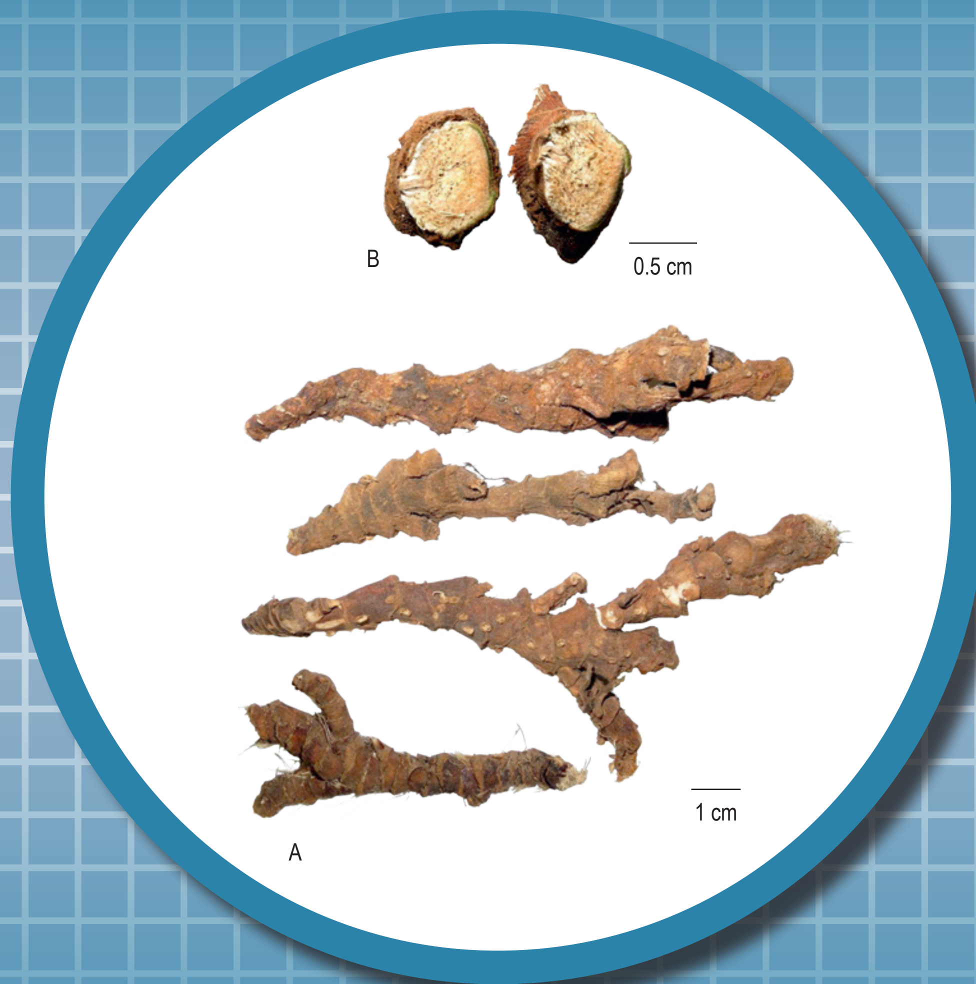

Figure 1 A photograph of Acori Tatarinowii Rhizoma

A. Acori Tatarinowii Rhizoma B. Transverse section

1. NAMES

Official Name: Acori Tatarinowii Rhizoma

Chinese Name: 石菖蒲

Chinese Phonetic Name: Shichangpu

2. SOURCE

Acori Tatarinowii Rhizoma is the dried rhizome of *Acorus tatarinowii* Schott (Araceae). The rhizome is collected in autumn and winter, fibrous roots and soil removed, then dried under the sun to obtain Acori Tatarinowii Rhizoma.

3. DESCRIPTION

Compressed-cylindrical, frequently tortuous, normally branched, 3-31 cm long, 3-16 mm in diameter. Externally brown or greyish-brown, rough, with uneven annulations, internodes 1-15 mm long, with fine longitudinal wrinkles, occasionally with remnants of fibrous roots or rounded root scars; leaf scars triangular, arranged alternately, some with hairy and scaly remnants of leaf bases. Texture hard. Fracture fibrous, white or pale red, an endodermis ring distinct, numerous dotted vascular bundles and brown oil spots visible. Odour aromatic; taste bitter and slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of brown cells with thickened outer wall. Cortex broad, scattered with numerous fibre bundles and leaf-trace vascular bundles. Endodermis distinct. Stele vascular bundles amphivasal or collateral, densely lined up near the endodermis, gradually larger and sparse inward, vascular bundles sheath distinct. Parenchyma scattered with subrounded secretory cells, the latter filled with secretions (Fig. 2).

Powder

Colour yellowish-brown. Fibres mostly in bundle, colourless or pale yellow, 6-10 μm in diameter, tapering towards the end, wall 2-7 μm thick, lignified, pit canals distinct. Fibre bundles surrounded by cells containing prisms of calcium oxalate, forming crystal fibres. Prisms of calcium oxalate polyhedral, subpolygonal or polyconelike, 4-13 μm in diameter; polychromatic under the polarized microscope. Simple starch granules ellipsoid, spheroidal or long-ovoid, 2-10 μm in diameter, hilum pointed, V-shaped or short slit-shaped, striation indistinct; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-20 (even more) units. Secretory cells abundant, subrounded or elongated-rounded, 14-43 μm in diameter, filled with orangish-yellow secretions. Epidermal cells of leaf sheath greyish-green or pale yellowish-brown, rectangular, 26-62 μm long, 9-31 μm wide. Vessels mainly reticulate, 9-32 μm in diameter (Fig. 3).

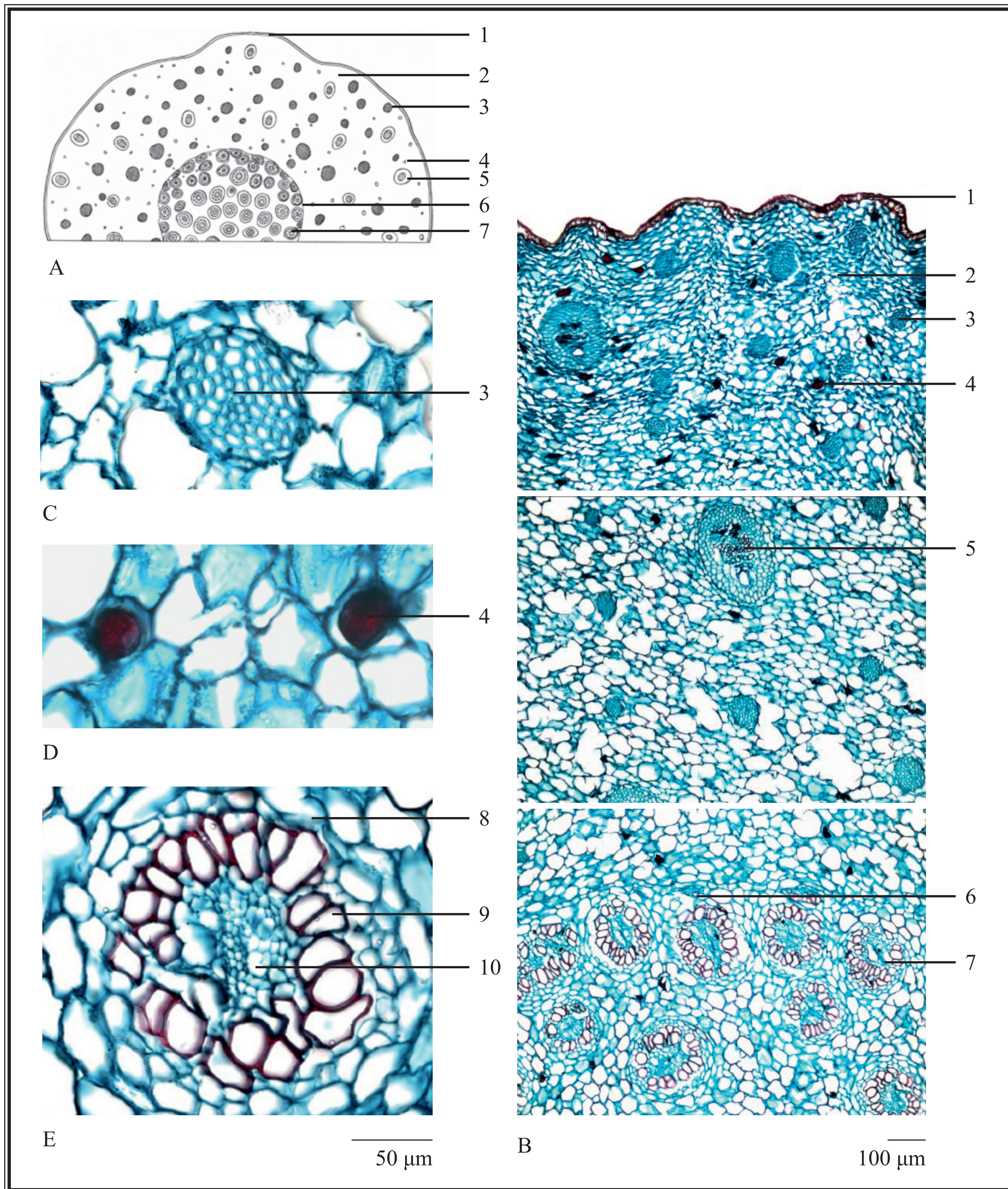

Figure 2 Microscopic features of transverse section of Acori Tatarinowii Rhizoma

A. Sketch B. Section illustration C. Fibre bundle D. Secretory cell
E. Stele vascular bundle

1. Epidermis 2. Cortex 3. Fibre bundle 4. Secretory cell
5. Leaf-trace vascular bundle 6. Endodermis 7. Stele vascular bundle
8. Vascular bundle sheath 9. Xylem 10. Phloem

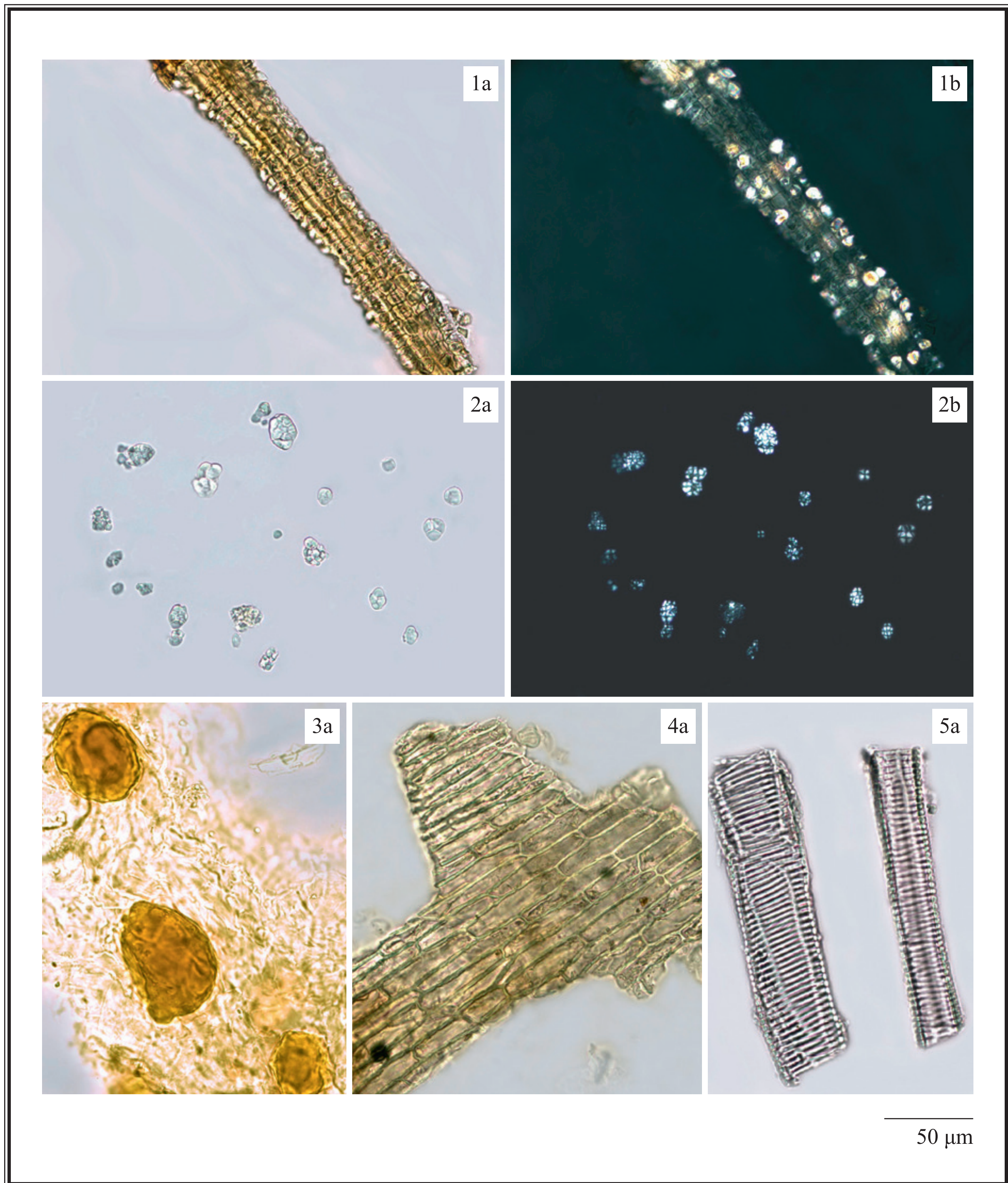

Figure 3 Microscopic features of powder of Acori Tatarinowii Rhizoma

1. Crystal fibres 2. Starch granules 3. Secretory cells
4. Epidermal cells of leaf sheath 5. Vessels

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

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

Standard solution

α-Asarone standard solution

Weigh 1.0 mg of α-asarone CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80˚C) and ethyl acetate (5:1, v/v).

Staining reagent

Iodine.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethyl acetate. Sonicate (100 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately α-asarone standard solution and the test solution (6 μL each) 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 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Fumigate the plate with iodine vapor chamber for about 30 min until the spots or bands become visible. Examine the plate under visible light. 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 α-asarone.

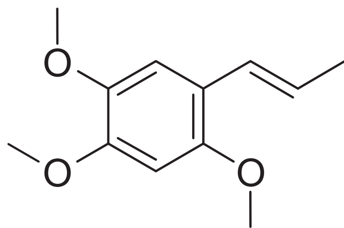

Figure 4 Chemical structure of α-asarone

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

Standard solution

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

Weigh 0.5 mg of α-asarone 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 centrifuge tube, then add 10 mL of ethanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with ethanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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 – 10	70 → 55	30 → 45	linear gradient
10 – 30	55	45	isocratic
30 – 40	55 → 25	45 → 75	linear gradient
40 – 60	25	75	isocratic

System suitability requirements

Perform at least five replicate injections, each using 8 μL of α-asarone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of α-asarone should not be more than 5.0%; the RSD of the retention time of α-asarone peak should not be more than 2.0%; the column efficiency determined from α-asarone peak should not be less than 20000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject α-asarone Std-FP and the test solution (8 μL each) into the HPLC system and record the chromatograms. Measure the retention time of α-asarone peak in the chromatogram of α-asarone Std-FP and the retention times of the two characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify α-asarone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of α-asarone Std-FP. The retention times of α-asarone 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 two characteristic peaks of Acori Tatarinowii Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the two characteristic peaks of Acori Tatarinowii Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (β-asarone)	0.89	± 0.03
2 (marker, α-asarone)	1.00	-

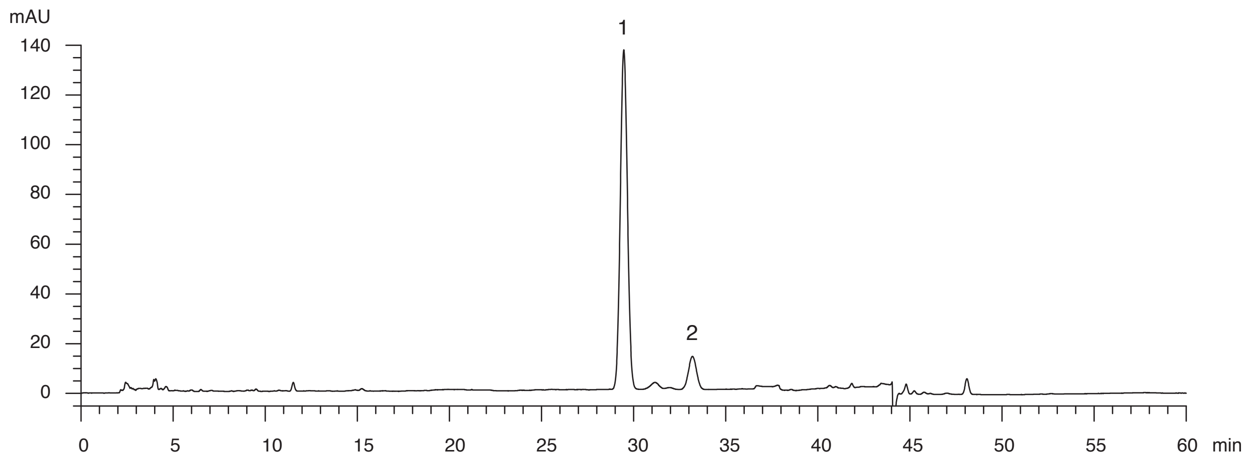

Figure 5 A reference fingerprint chromatogram of Acori Tatarinowii Rhizoma extract

For positive identification, the sample must give the above two 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 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 9.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 16.0%.
Ethanol-soluble extractives (hot extraction method): not less than 7.0%.

7. ASSAY

7.1 Assay of α-asarone

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

Standard solution

α-Asarone standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 2.0 mg of α-asarone CRS and dissolve in 2 mL of ethanol.
α-Asarone standard solution for assay, Std-AS
Measure accurately the volume of the α-asarone Std-Stock, dilute with ethanol to produce a series of solutions of 0.5, 1, 30, 60, 90 mg/L for α-asarone.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants and make up to the mark with ethanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (257 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 a mixture of water and acetonitrile (55:45, v/v). The elution time is about 35 min.

System suitability requirements

Perform at least five replicate injections, each using 8 µL of α-asarone Std-AS (30 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of α-asarone should not be more than 5.0%; the RSD of the retention time of α-asarone peak should not be more than 2.0%; the column efficiency determined from α-asarone peak should not be less than 10000 theoretical plates.

The *R* value between α-asarone 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 α-asarone Std-AS (8 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of α-asarone against the corresponding concentrations of α-asarone Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 8 µL of the test solution into the HPLC system and record the chromatogram. Identify α-asarone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of α-asarone Std-AS. The retention times of α-asarone 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 α-asarone in the test solution, and calculate the percentage content of α-asarone in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.076% of α-asarone ($C_{12}H_{16}O_3$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 20 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 300 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method B).

Limits

The sample contains not less than 1.0% (v/w) of volatile oil.

8. Caution

This CMM should only be used in decoction after boiling for 1 h.