Sarcandrae Herba

Figure 1 A photograph of Sarcandrae Herba

A. Sarcandrae Herba B. Magnified stem C. Magnified upper surface of leaf
D. Magnified lower surface of leaf

1. NAMES

Official Name: Sarcandrae Herba

Chinese Name: 腫節風

Chinese Phonetic Name: Zhongjiefeng

2. SOURCE

Sarcandrae Herba is the dried herb of *Sarcandra glabra* (Thunb.) Nakai (Chloranthaceae). The herb is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Sarcandrae Herba.

3. DESCRIPTION

40-100 cm long. Rhizomes relatively large, with numerous rootlets. Stems cylindrical, usually branched, 3-13 mm in diameter; externally dark green to dark brown, with distinct fine longitudinal striations, longitudinal lenticels scattered, nodes swollen; texture fragile, easily broken; fracture with pith or hollow. Leaves opposite, lamina ovate-lanceolate to ovate-elliptical, 4-12 cm long, 3-6 cm wide; externally dark brown or brownish-red, glabrous, margins roughly serrate, the tips of serrations with blackish-brown glandular bodies, petiole about 1 cm long; texture nearly leathery. Odour slightly aromatic; taste slightly pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Outer walls of epidermal cells thickened, brownish-black. Cortex broad, consisting of parenchymatous cells, parenchymatous cells contain starch granules. Phloem narrow. Cambium distinct. Xylem completely lignified [Fig. 2 (i)].

Rhizome: Epidermal cells subrectangular or oblong, covered with cuticle, edge obtusely dentate. Cortex consists of 10 or more layers of cells; the outer 2-3 layers composed of collenchymatous cells; parenchymatous cells located in the inner part, containing brownish-yellow contents; stone cells scattered singly or in groups. Pericyclic fibre bundles crescent, arranged in an interrupted ring, lignified. Phloem narrow. Xylem consists of vessels and tracheids, rays 2-8 cells wide. Parenchymatous cells of pith relatively large, stone cells sometimes visible, scattered singly or in groups [Fig. 2 (ii)].

Stem: Similar to the Rhizome. Differences: Less xylem, pith bigger [Fig. 2 (iii)].

Leaf: Epidermal cells square or rectangular, covered with thick cuticle, stomata occasionally found on the lower epidermis. Collenchymatous cells located at the inner side of upper and lower epidermis at the vascular bundle of midvein. Mesophyll tissue consists of 1 layer of palisade tissue and spongy tissue, arranged loosely. Vascular bundles of midrib collateral, fibre bundles surrounded. Stone cells irregularly polygonal, scattered singly or in groups [Fig. 2 (iv)].

Powder

Colour yellowish-green to greenish-brown. Xylem parenchymatous cells subsquare or rectangular, containing brownish-yellow contents. Epidermal cells of leaf square or rectangular, anticlinal walls slightly sinuous or relatively straight, stomata anomocytic, subsidiary cells 3-5. Stone cells scattered singly or in groups, subsquare, subrounded or irregular polygonal, 40-60 μm in diameter; white or yellowish-white under the polarized microscope. Fibres mostly in bundles; yellow under the polarized microscope. Tracheids long and narrow, fusiform or long slat-shaped, 6-30 μm in diameter; bright white under the polarized microscope. Vessels mainly reticulate, spiral and annular, easily observe (Fig. 3).

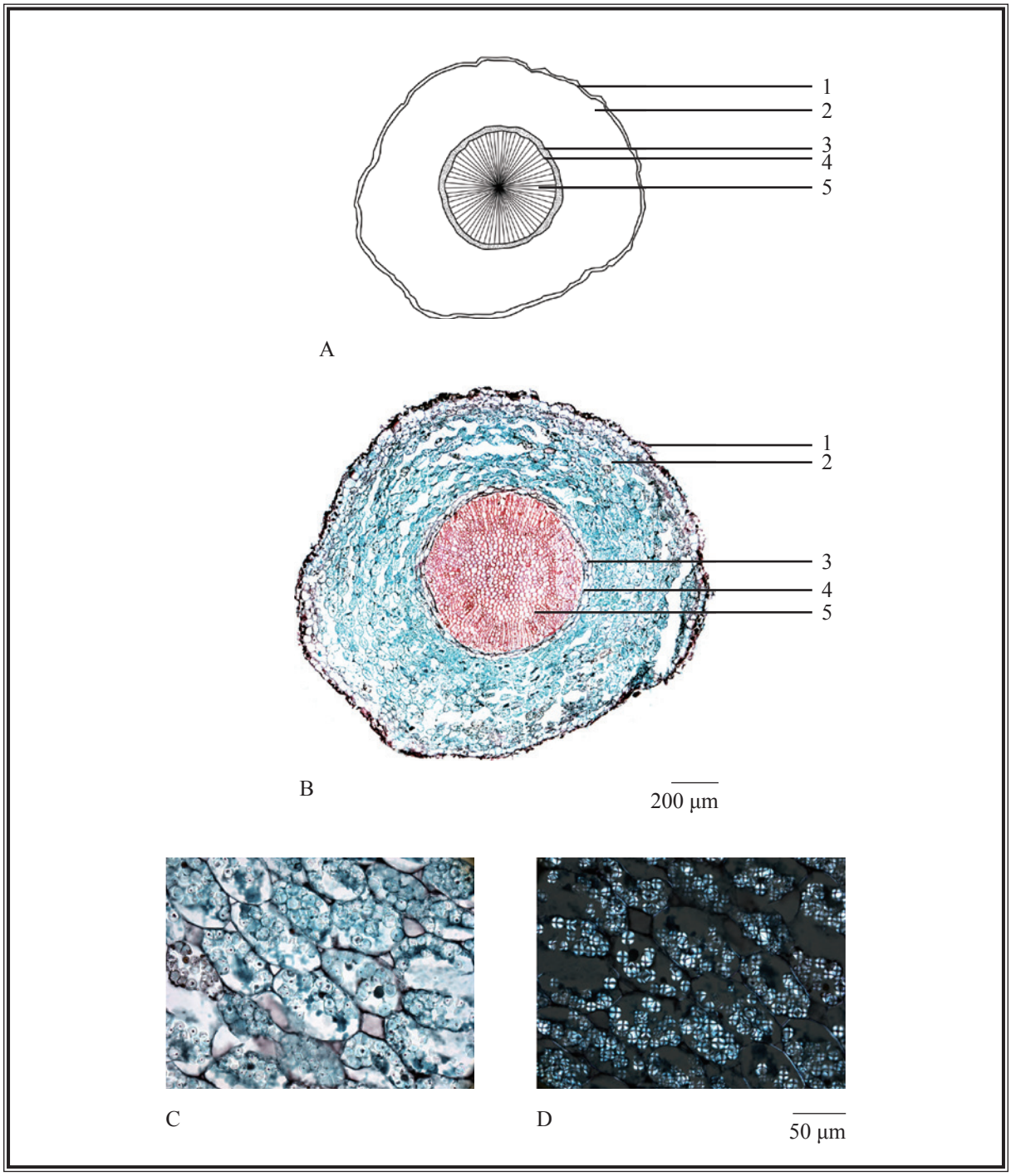

Figure 2 (i) Microscopic features of transverse section of root of Sarcandrae Herba

A. Sketch B. Section illustration C. Starch granules
D. Starch granules (under polarized microscope)

1. Epidermis 2. Cortex 3. Phloem 4. Cambium 5. Xylem

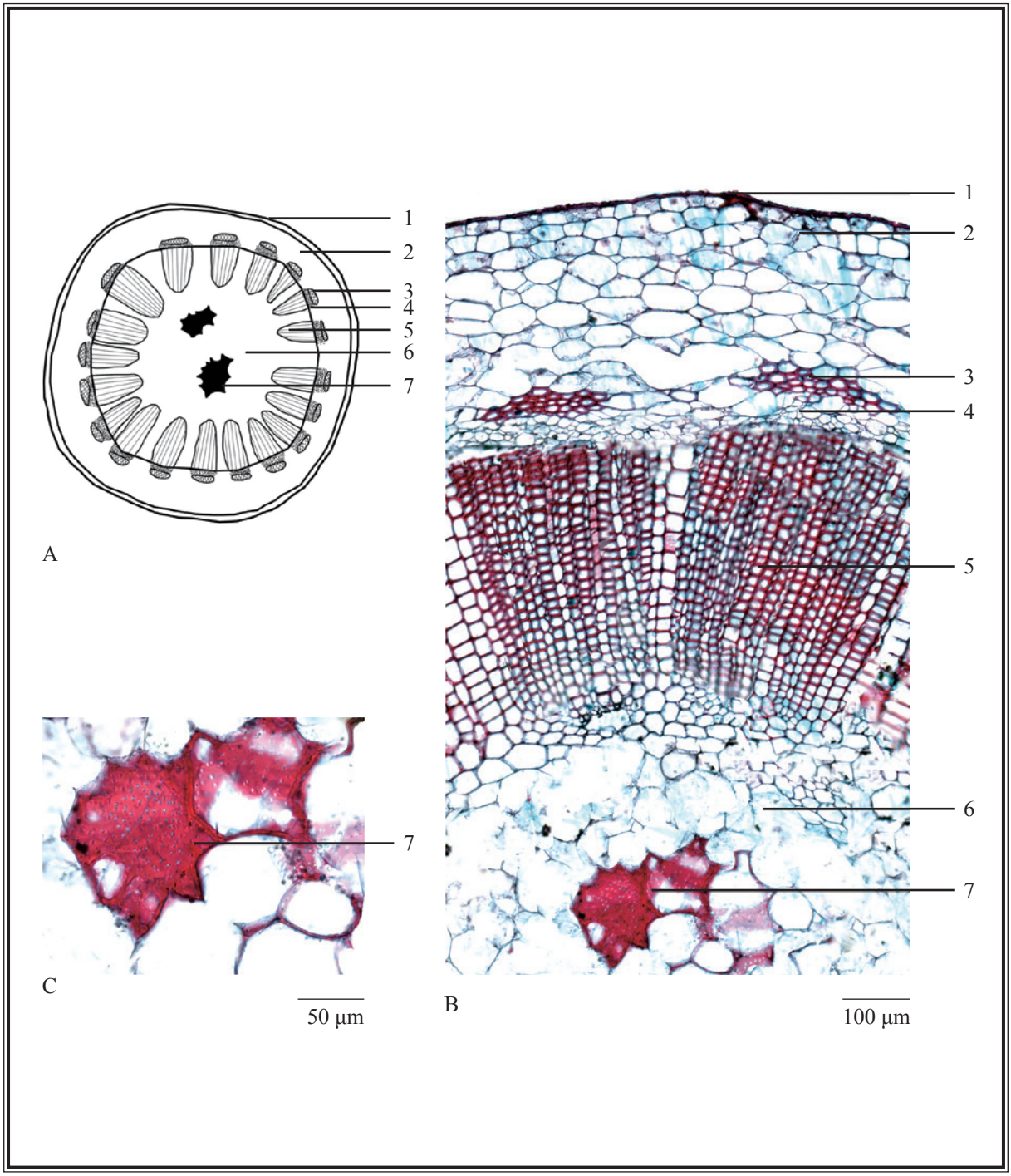

Figure 2 (ii) Microscopic features of transverse section of rhizome of Sarcandrae Herba

A. Sketch B. Section illustration C. Stone cells

1. Epidermis 2. Cortex 3. Pericyclic fibre bundles 4. Phloem 5. Xylem 6. Pith 7. Stone cell

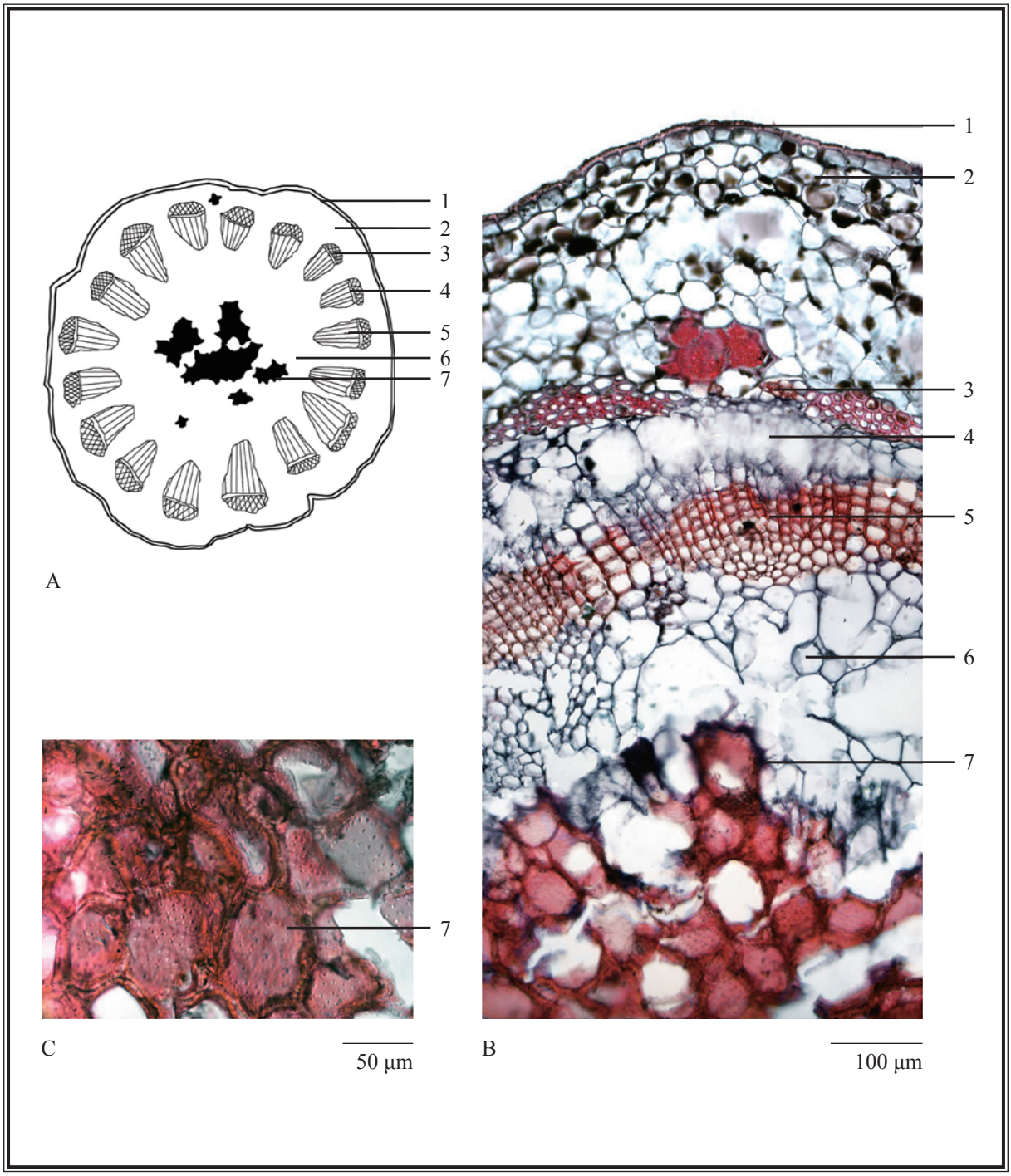

Figure 2 (iii) Microscopic features of transverse section of stem of Sarcandrae Herba

A. Sketch B. Section illustration C. Stone cells

1. Epidermis 2. Cortex 3. Pericyclic fibre bundles 4. Phloem 5. Xylem 6. Pith 7. Stone cell

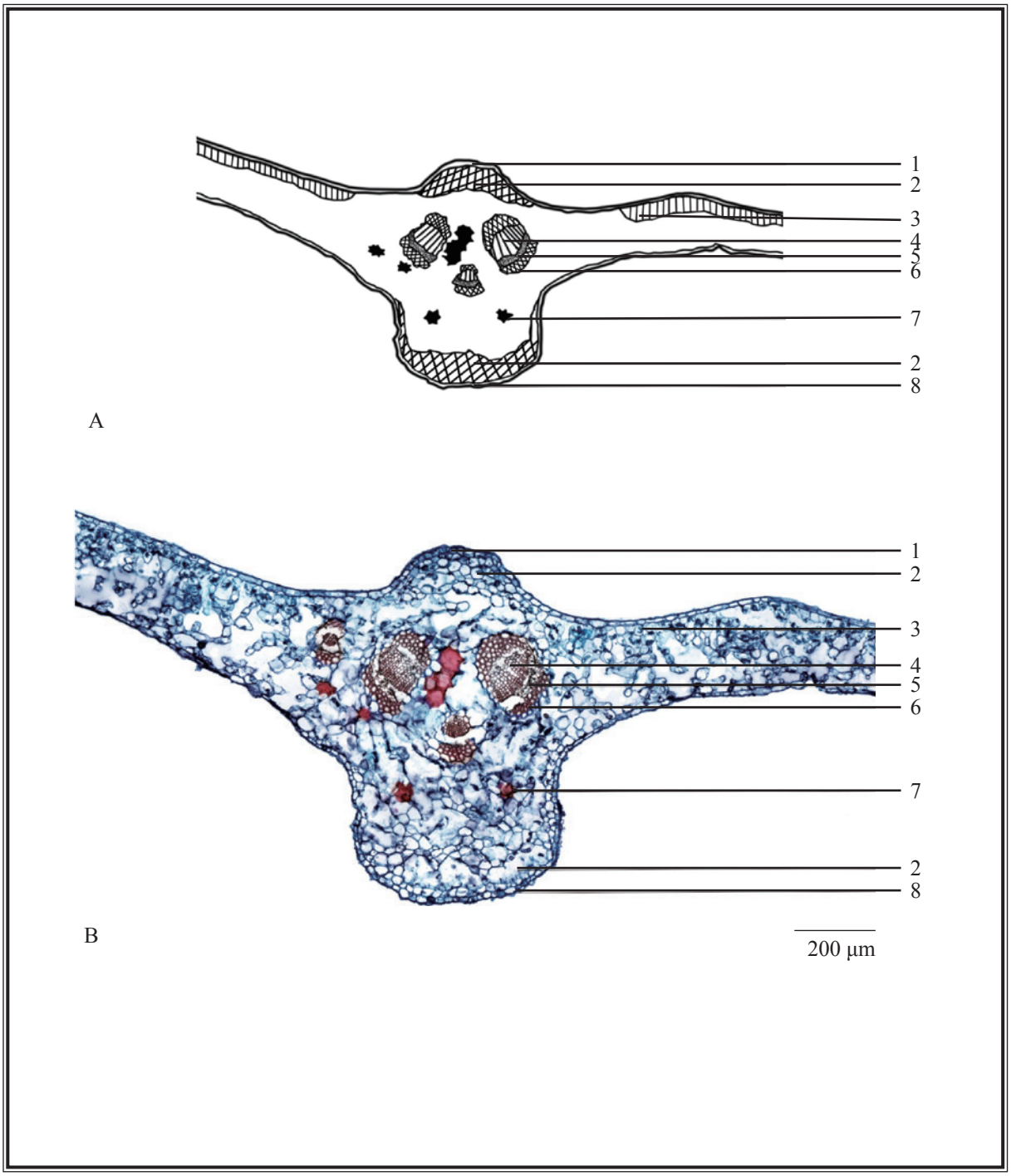

Figure 2 (iv) Microscopic features of transverse section of leaf of Sarcandrae Herba

A. Sketch B. Section illustration

1. Upper epidermis 2. Collenchyma 3. Mesophyll tissue 4. Xylem 5. Phloem
6. Fibre bundles 7. Stone cells 8. Lower epidermis

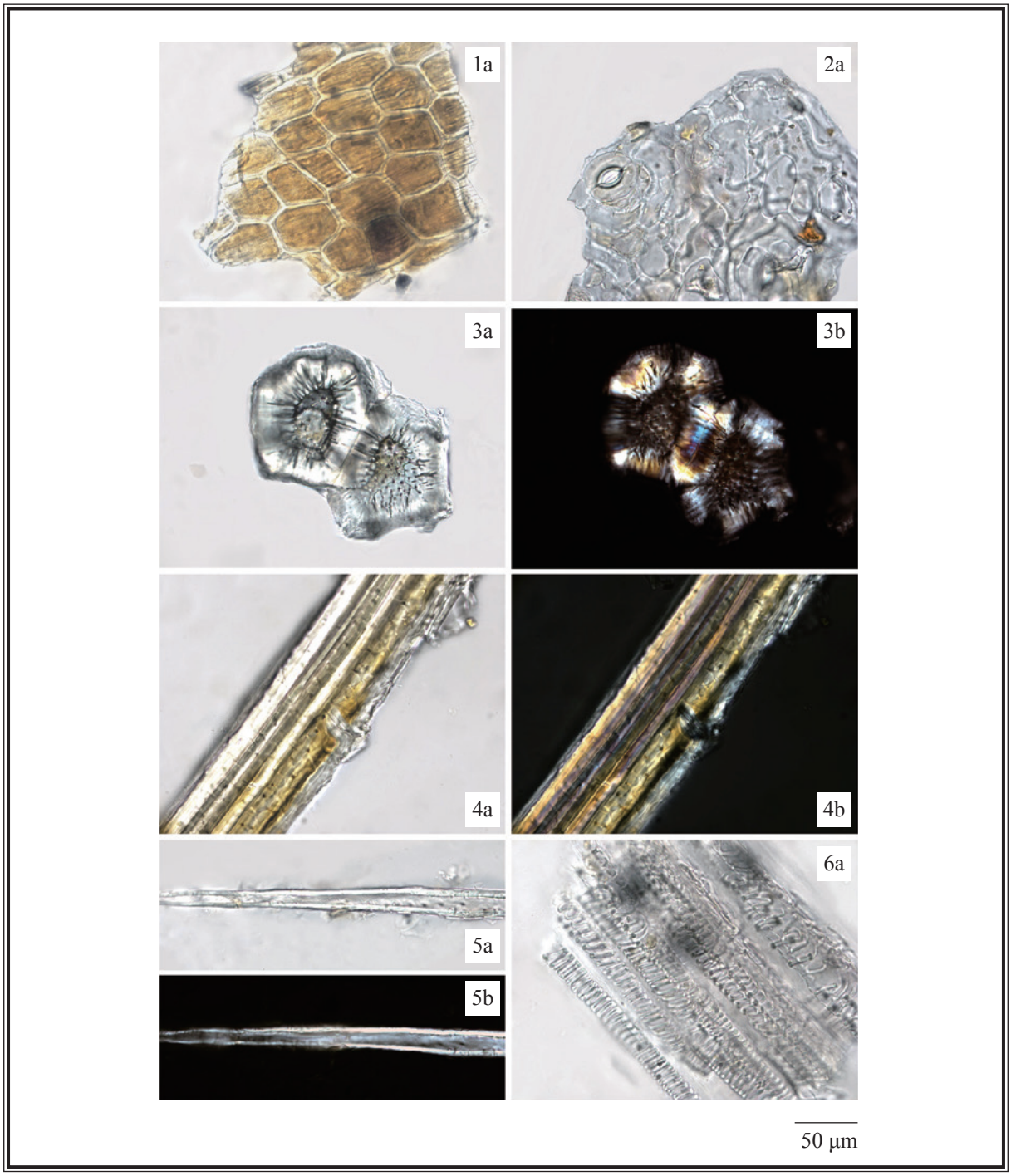

Figure 3 Microscopic features of powder of Sarcandrae Herba

1. Xylem parenchymatous cells 2. Epidermal cells of leaf 3. Stone cells
4. Fibre bundle 5. Tracheid 6. Vessels

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

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

Standard solutions

Isofraxidin standard solution

Weigh 0.1 mg of isofraxidin CRS (Fig. 4) and dissolve in 1 mL of methanol (70%).

Rosmarinic acid standard solution

Weigh 0.3 mg of rosmarinic acid CRS (Fig. 4) and dissolve in 1 mL of methanol (70%).

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and formic acid (7:7:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol (70%). Sonicate (100 W) the mixture for 15 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 (70%).

Procedure

Carry out the method by using a TLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately isofraxidin standard solution (3 μL), rosmarinic acid standard solution (5 μL) and the test solution (5 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC plate for development. Develop over a path of about 7.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

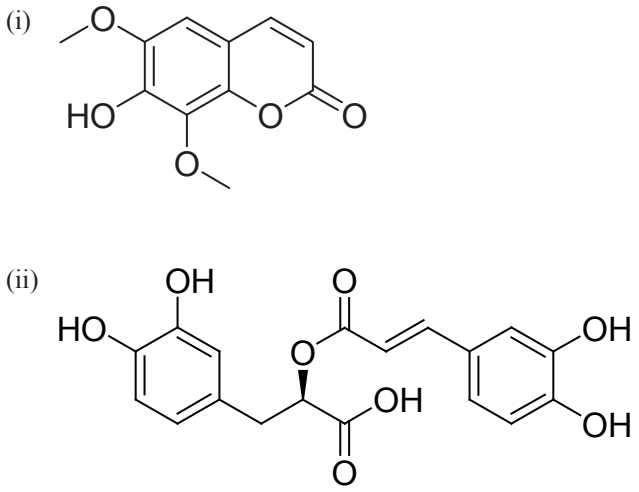

Figure 4 Chemical structures of (i) isofraxidin and (ii) rosmarinic acid

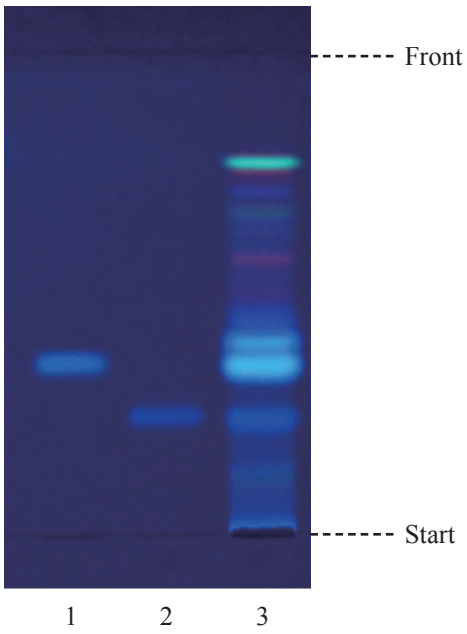

Figure 5 A reference TLC chromatogram of Sarcandrae Herba extract observed under UV light (366 nm)

1. Isofraxidin standard solution 2. Rosmarinic acid standard solution 3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of isofraxidin and rosmarinic acid (Fig. 5).

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

Standard solutions

Isofraxidin standard solution for fingerprinting, Std-FP (24 mg/L)

Weigh 1.2 mg of isofraxidin CRS and dissolve in 50 mL of methanol.

Rosmarinic acid standard solution for fingerprinting, Std-FP (24 mg/L)

Weigh 1.2 mg of rosmarinic acid CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 25 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Wash the residue with methanol. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-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 (342 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	90 → 85	10 → 15	linear gradient
25 – 40	85 → 80	15 → 20	linear gradient
40 – 60	80 → 70	20 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of isofraxidin Std-FP and rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isofraxidin and rosmarinic acid should not be more than 5.0%; the RSD of the retention times of isofraxidin and rosmarinic acid peaks should not be more than 2.0%; the column efficiencies determined from isofraxidin and rosmarinic acid peaks should not be less than 40000 and 150000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and 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. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.43	± 0.03
2	0.55	± 0.03
3 (marker, isofraxidin)	1.00	-
4 (rosmarinic acid)	1.42	± 0.03

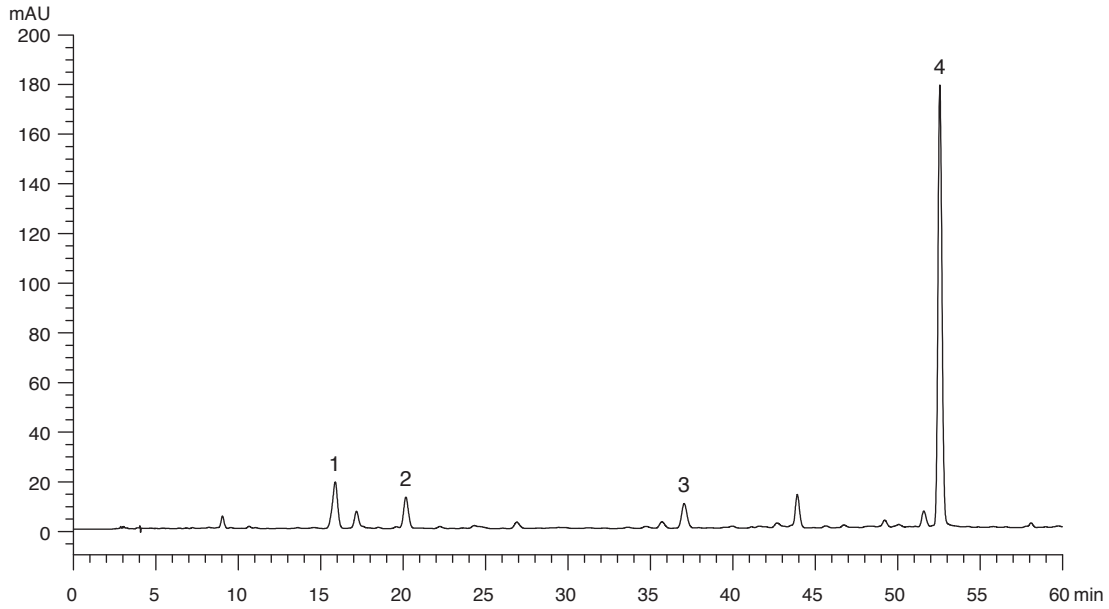

Figure 6 A reference fingerprint chromatogram of Sarcandrae Herba extract

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 7.5%.
Acid-insoluble ash: not more than 1.5%.

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 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

Mixed isofraxidin and rosmarinic acid standard stock solution, Std-Stock (460 mg/L each)

Weigh accurately 2.3 mg of isofraxidin CRS and 2.3 mg of rosmarinic acid CRS, and dissolve in 5 mL of methanol.

Mixed isofraxidin and rosmarinic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed isofraxidin and rosmarinic acid Std-Stock, dilute with methanol to produce a series of solutions of 4.6, 9.2, 23, 46, 69 mg/L for both isofraxidin and rosmarinic acid.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 25 mL of methanol. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Wash the residue with methanol. Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-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 (342 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 25	83	17	isocratic
25 – 40	83 → 70	17 → 30	linear gradient
40 – 50	70	30	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed isofraxidin and rosmarinic acid Std-AS (23 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isofraxidin and rosmarinic acid should not be more than 5.0%; the RSD of the retention times of isofraxidin and rosmarinic acid peaks should not be more than 2.0%; the column efficiencies determined from isofraxidin and rosmarinic acid peaks should not be less than 10000 and 100000 theoretical plates respectively.

The *R* value between isofraxidin peak and the closest peak; and the *R* value between rosmarinic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed isofraxidin and rosmarinic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of isofraxidin and rosmarinic acid against the corresponding concentrations of the mixed isofraxidin and rosmarinic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify isofraxidin and rosmarinic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed isofraxidin and rosmarinic acid Std-AS. The retention times of isofraxidin and rosmarinic acid peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of isofraxidin and rosmarinic acid in the test solution, and calculate the percentage contents of isofraxidin and rosmarinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.025% of isofraxidin ($C_{11}H_{10}O_5$) and not less than 0.020% of rosmarinic acid ($C_{18}H_{16}O_8$), calculated with reference to the dried substance.