Valerianae Radix et Rhizoma

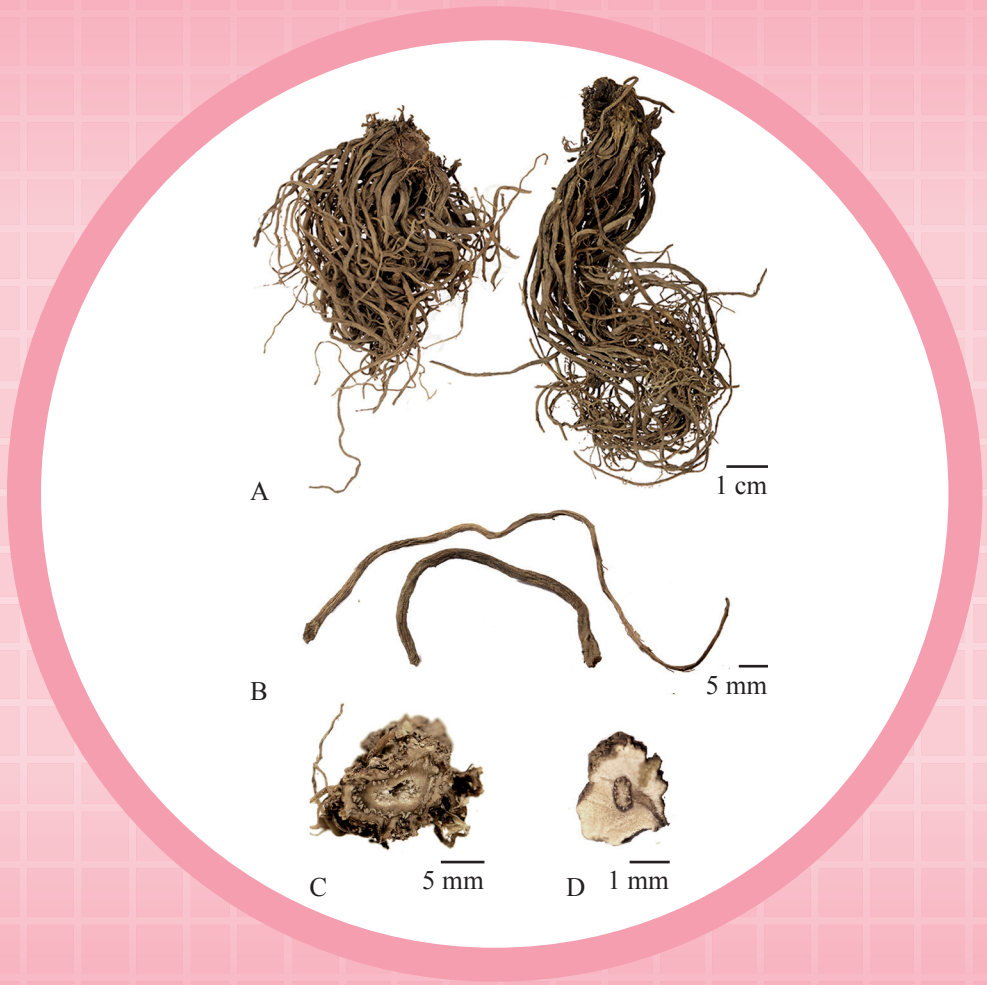

Figure 1 A photograph of Valerianae Radix et Rhizoma

A. Valerianae Radix et Rhizoma B. Magnified image of root
C. Magnified image of cut surface of rhizome
D. Magnified image of cut surface of root

1. NAMES

Official name: Valerianae Radix et Rhizoma

Chinese name: 纈草

Chinese phonetic name: Xiecao

2. SOURCE

Valerianae Radix et Rhizoma is the dried root and rhizome of *Valeriana officinalis* L. (Valerianaceae). The root and rhizome are collected during September to October, foreign matter removed, then dried under the sun to obtain Valerianae Radix et Rhizoma.

3. DESCRIPTION

Rhizome obconical or cylindrical, short and horny, 4-22 mm in diameter; externally dull yellowish-brown to dark brown, with remnants of stem bases and leaf scars; usually entirely covered by numerous roots; fracture surface greyish-brown, irregular, sometimes hollow. Root almost cylindrical, longitudinally striated, 2-14 cm long, 1-3 mm in diameter; externally almost the same colour of the rhizome, slender, with fibrous lateral rootlets; fracture surface greyish-white, showing a narrow central stele. Texture brittle. Odour strong and characteristic; taste sweetish initially, becoming camphoraceous and somewhat bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Epidermis consists of 1 layer of cells, outer cell walls convex. Hypodermal cells 1 layer, comparatively large, polygonal or subrounded, walls slightly suberized; cortex broad, parenchymatous cells spheroidal or polygonal. Oil cells numerous, scattered in cortex, smaller than cortex parenchymatous cells. Endodermis consists of 1 layer of tangentially elongated cells. Sclerenchymatous cells occasionally singly scattered or several arranged in an interrupted ring outside endodermis. Vascular bundle collateral, arranged in a ring and surrounding the pith; phloem cells irregular in shape; cambium indistinct; vessels and fibres lignified. Pith small, clefts occasionally present [Fig. 2 (i)].

Rhizome: Cork consists of several layers of cells, cells polygonal, comparatively large, and slightly suberized. Cortex cells several layers, spheroidal, polygonal or irregular in shape, walls slightly thickened. Endodermis consists of 1 layer of tangentially elongated cells. Wedge-shaped vascular bundles collateral, circularly arranged; phloem cells irregular in shape, loosely arranged; cambium indistinct; vessels and fibres lignified. Oil cells occasionally scattered in parenchyma. Pith relatively large, occasionally with clefts; stone cells present in the centre, with thick pitted walls and narrow lumen [Fig. 2 (ii)].

Powder

Colour greyish-yellow or greyish-brown. Starch granules abundant, 4-16 μm in diameter, with cleft or stellate hilum, the compound granules composed of 2-5 units; black and cruciate-shaped under the polarized microscope. Stone cells single or in groups, polygonal, with thick walls and narrow lumen; polychromatic under the polarized microscope. Cork cells polygonal, with irregularly thickened walls. Oil cells infrequently observed, scattered among parenchymatous cells. Hypodermal cells rectangular to polygonal, slightly thickened and elongated. Sclerenchymatous cells occasionally observed, rectangular, with moderately thickened wall and large lumen; polychromatic under the polarized microscope. Fibres mostly in bundles, pale yellow, with lignified cell walls and distinct pits, slender, 6-26 μm in diameter; polychromatic under the polarized microscope. Endodermis cells rectangular, showing sinuous tangential walls. Bordered-pitted or reticulate vessels 6-66 μm in diameter (Fig. 3).

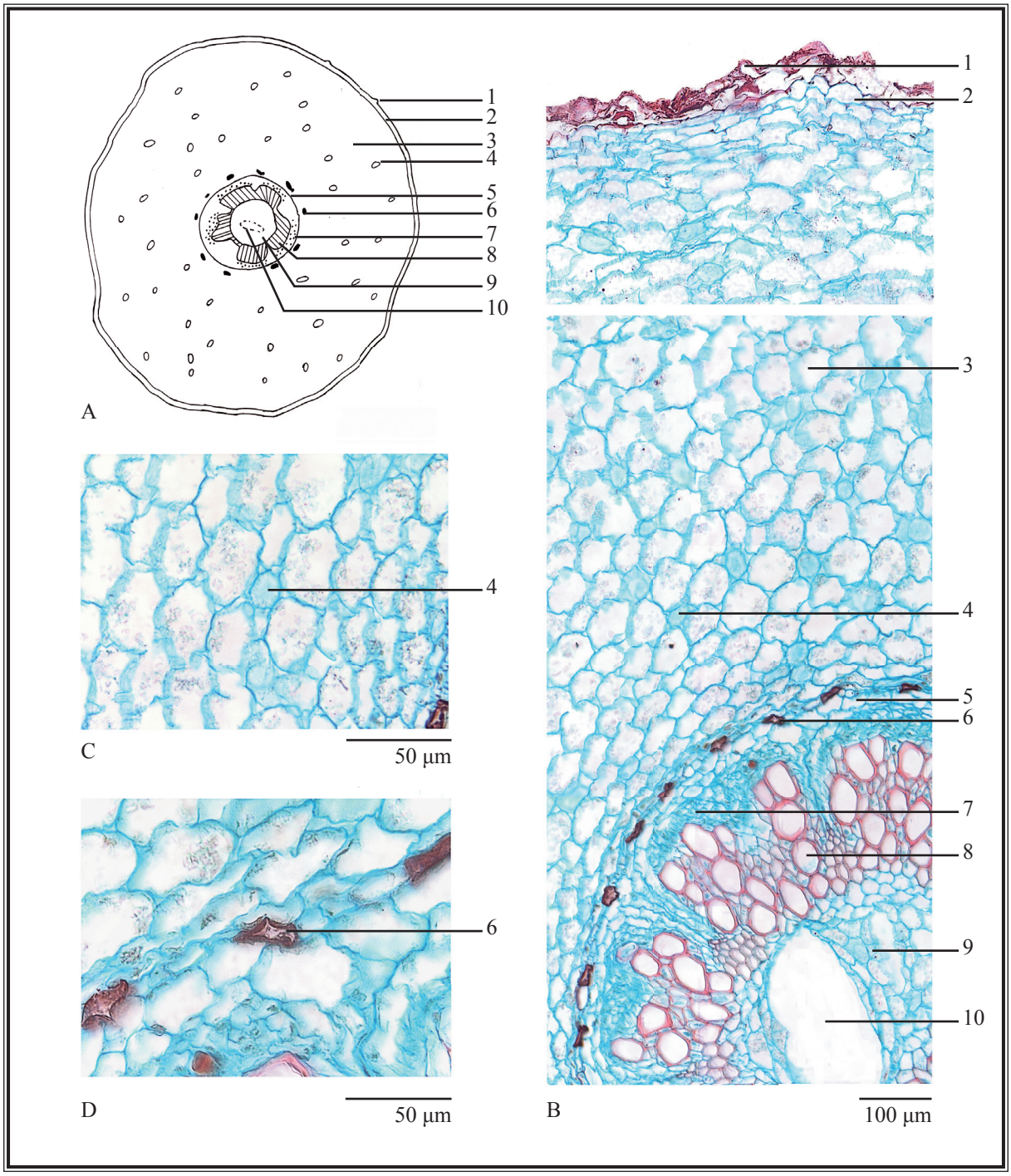

Figure 2 (i) Microscopic features of transverse section of root of Valerianae Radix et Rhizoma

A. Sketch B. Section illustration C-D. Section magnified

1. Epidermis 2. Hypodermis 3. Cortex 4. Oil cell 5. Endodermis
6. Sclerenchymatous cell 7. Phloem 8. Xylem 9. Pith 10. Cleft

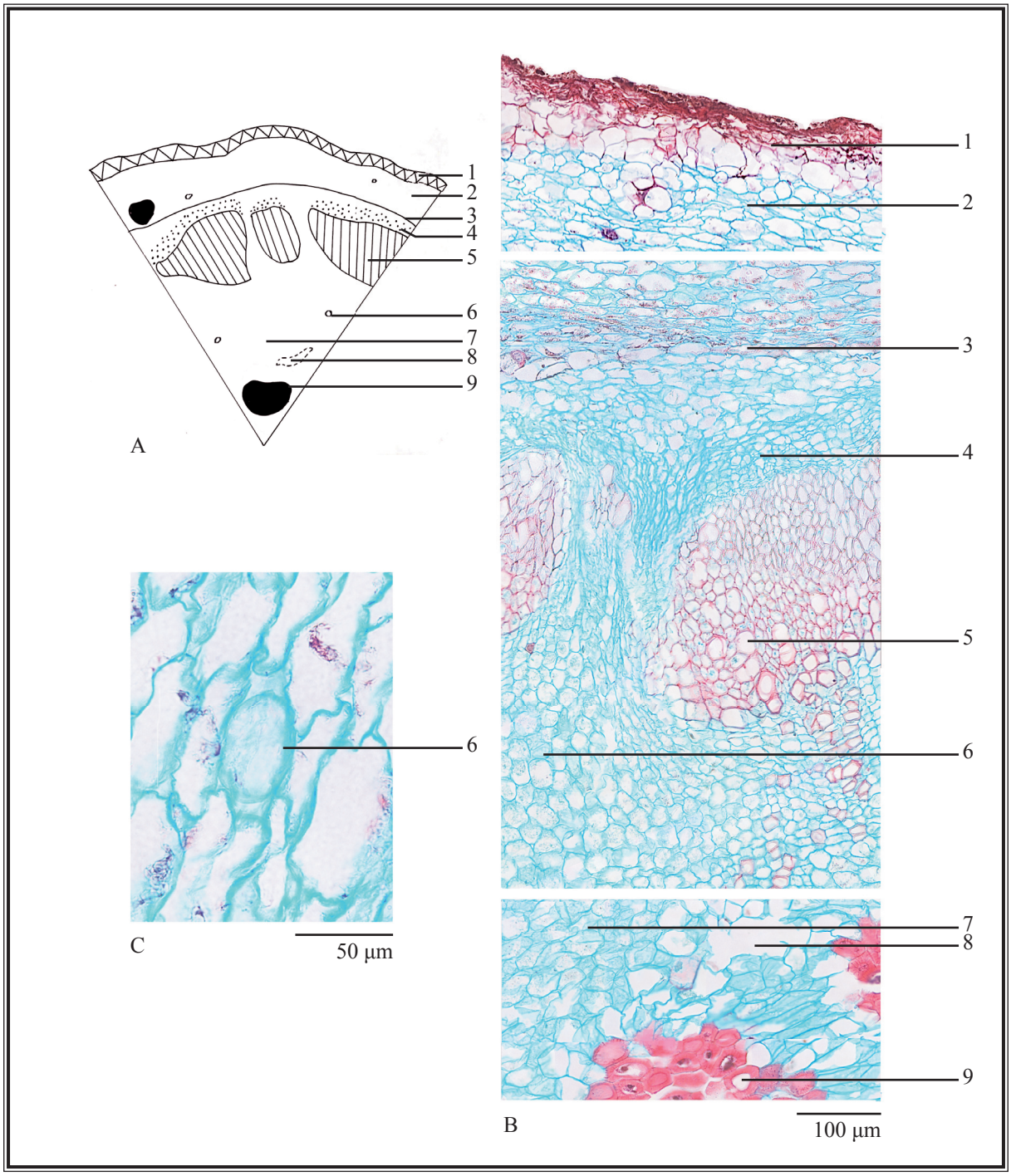

Figure 2 (ii) Microscopic features of transverse section of rhizome of Valerianae Radix et Rhizoma

A. Sketch B. Section illustration C. Oil cell

1. Cork 2. Cortex 3. Endodermis 4. Phloem 5. Xylem 6. Oil cell 7. Pith
8. Cleft 9. Stone cell

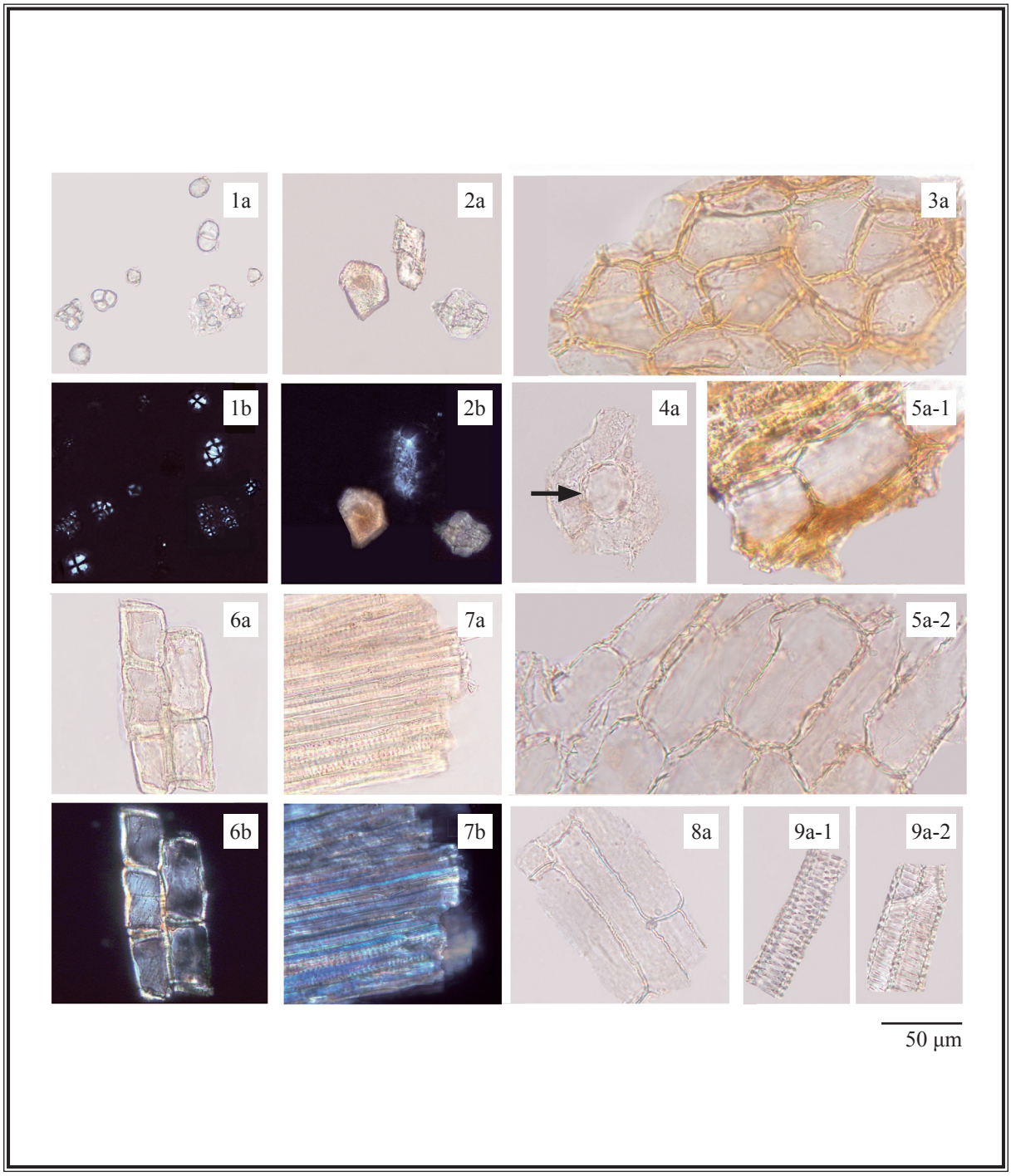

Figure 3 Microscopic features of powder of Valerianae Radix et Rhizoma

1. Starch granules 2. Stone cells 3. Cork cells 4. Oil cell (→)
5. Hypodermal cells (5-1 lateral view, 5-2 surface view)
6. Sclerenchymatous cells 7. Fibres 8. Endodermal cells
9. Vessels (9-1 bordered-pitted vessel, 9-2 reticulate vessels)

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

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

Standard solution

Valerenic acid standard solution

Weigh 0.5 mg of valerenic acid CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and formic acid (8:2:0.2, v/v).

Spray reagent

Spray reagent 1

Add slowly 10 mL of glacial acetic acid to 40 mL of hydrochloric acid.

Spray reagent 2

Mix 0.5 mL of *p*-anisaldehyde into 10 mL of glacial acetic acid. Add 85 mL of methanol. Add cautiously 5 mL of sulphuric acid.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ethanol (70%). Sonicate (400 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 valerenic acid standard solution and the test solution (5 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 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 1 and heat at about 120ºC (about 5 min). Spray the plate evenly with the spray reagent 2 and heat at about 100ºC until the spots or bands become visible (about 3 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

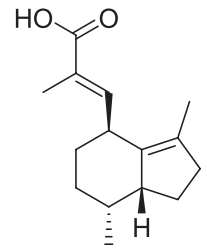

Figure 4 Chemical structure of valerenic acid

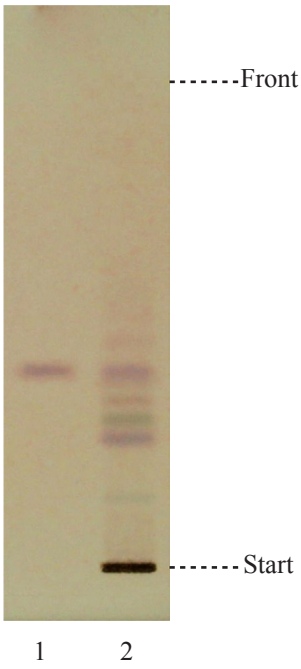

Figure 5 A reference HPTLC chromatogram of Valerianae Radix et Rhizoma extract observed under visible light after staining

1. Valerenic acid 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 valerenic acid (Fig. 5).

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

Standard solution

Valerenic acid standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 1.5 mg of valerenic acid CRS and dissolve in 100 mL of ethanol (70%).

Test solution

Weigh 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Wash the residue with ethanol (70%). Combine the extracts and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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 – 15	50 → 40	50 → 60	linear gradient
15 – 25	40 → 30	60 → 70	linear gradient
25 – 35	30	70	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of valerenic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of valerenic acid should not be more than 5.0%; the RSD of the retention time of valerenic acid peak should not be more than 2.0%; the column efficiency determined from valerenic acid peak should not be less than 24000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject valerenic acid Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention time of valerenic acid peak in the chromatogram of valerenic acid Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify valerenic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of valerenic acid Std-FP. The retention times of valerenic acid 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 Valerianae Radix et Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Valerianae Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.52	± 0.03
2 (marker, valerenic acid)	1.00	-
3	1.11	± 0.03
4	1.47	± 0.03

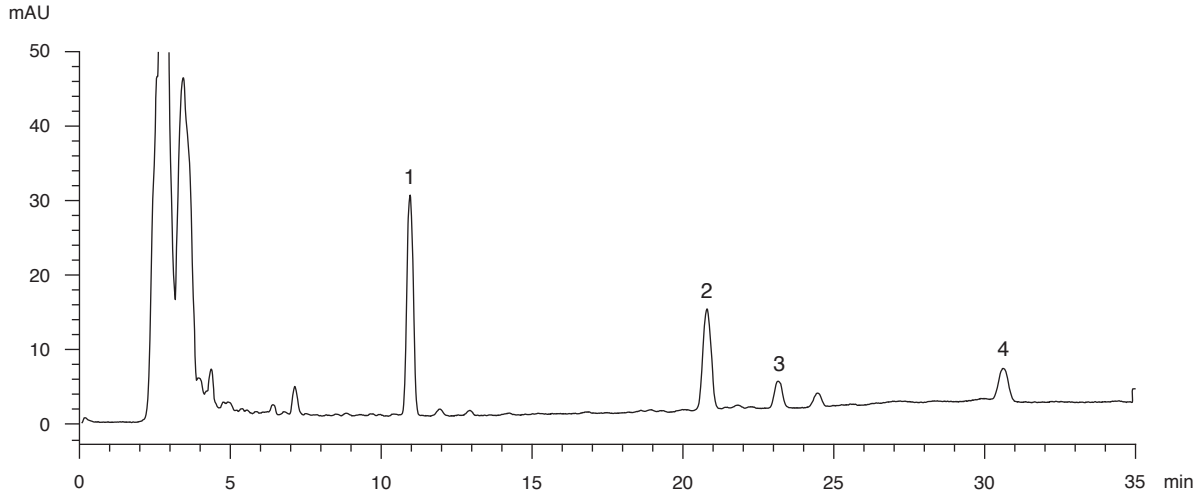

Figure 6 A reference fingerprint chromatogram of Valerianae Radix et Rhizoma extract

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 XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.5%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 25.0%.
Ethanol-soluble extractives (cold extraction method): not less than 25.0%.

7. ASSAY

7.1 Assay of Valerenic acid

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

Standard solution

Valerenic acid standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of valerenic acid CRS and dissolve in 10 mL of ethanol (70%).
Valerenic acid standard solution for assay, Std-AS
Measure accurately the volume of the valerenic acid Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 5, 10, 15, 20, 30 mg/L for valerenic acid.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (70%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Wash the residue with ethanol (70%). Combine the extracts and make up to the mark with ethanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (220 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 – 15	50 → 40	50 → 60	linear gradient
15 – 25	40 → 30	60 → 70	linear gradient
25 – 35	30	70	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of valerenic acid Std-AS (15 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of valerenic acid should not be more than 5.0%; the RSD of the retention time of valerenic acid peak should not be more than 2.0%; the column efficiency determined from valerenic acid peak should not be less than 24000 theoretical plates.

The *R* value between valerenic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of valerenic acid Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of valerenic acid against the corresponding concentrations of valerenic acid Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify valerenic acid peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of valerenic acid Std-AS. The retention times of valerenic acid 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 valerenic acid in the test solution, and calculate the percentage content of valerenic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.093% of valerenic acid ($C_{15}H_{22}O_2$), calculated with reference to the dried substance.

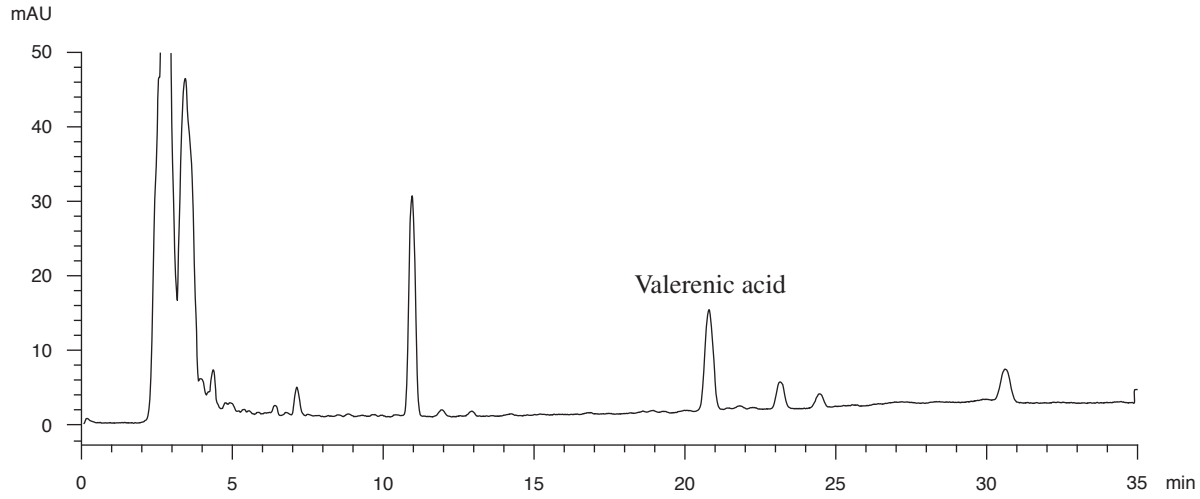

Figure 7 A reference assay chromatogram of Valerianae Radix et Rhizoma extract

7.2 Assay of Volatile Oil

Weigh accurately 100 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 500 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 0.50% (v/w) of volatile oil.