Rubiae Radix et Rhizoma

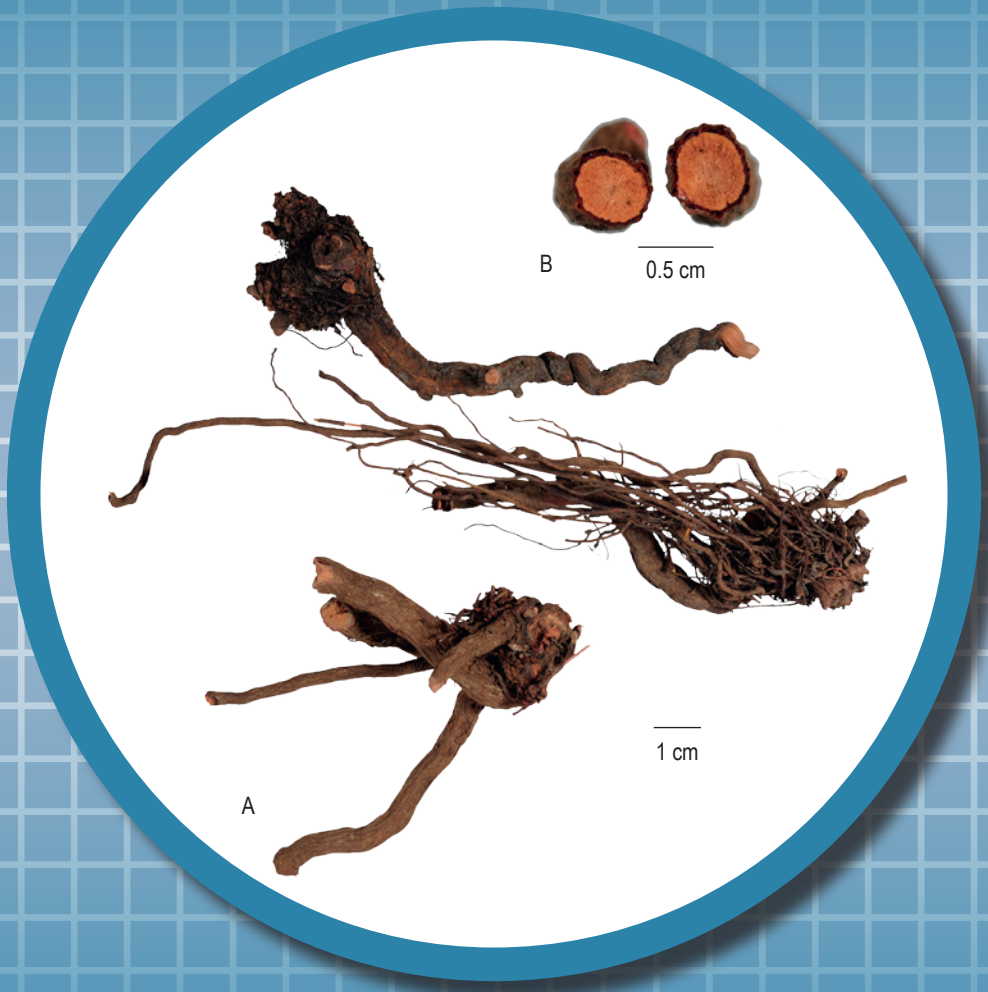

Figure 1 A photograph of Rubiae Radix et Rhizoma

A. Rubiae Radix et Rhizoma B. Magnified transverse section of roots

1. NAMES

Official Name: Rubiae Radix et Rhizoma

Chinese Name: 茜草

Chinese Phonetic Name: Qiancao

2. SOURCE

Rubiae Radix et Rhizoma is the dried root and rhizome of *Rubia cordifolia* L. (Rubiaceae). The root and rhizome are collected in spring and autumn, soil removed, washed clean, then dried under the sun to obtain Rubiae Radix et Rhizoma.

3. DESCRIPTION

Rhizomes nodular, with numerous fascicled roots varying in thickness. Roots cylindrical, slightly curved, 3.5-24 cm long, 1-10 mm in diameter, externally reddish-brown or dark brown, with fine longitudinal wrinkles and a few rootlet scars; the area where the outer bark has exfoliated appearing yellowish-red. Texture fragile, easily broken. Fracture even, bark narrow and purplish-red; xylem broad, pale yellowish-red, showing numerous pores of vessels. Odour slight; taste bitter and irritative to tongue when chewed continually (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: Cork consists of 6-12 layers of cells, containing brown contents. Cortex narrow, consisting of 4-5 layers of cells. Phloem cells relatively small. Cambium in a ring, less indistinct. Xylem occupies the major portion of the root, completely lignified; xylem rays indistinct. Parenchymatous cells contain raphides of calcium oxalate [Fig. 2 (i)].

Rhizome: Cork consists of 6-12 layers of cells, containing brown contents. Cortex narrow, consisting of 4-5 layers of cells. Phloem cells relatively small. Cambium in a ring, indistinct. Xylem occupies the greater portion of the rhizome, completely lignified, xylem rays indistinct. Parenchymatous cells contain raphides of calcium oxalate. Pith consists of parenchymatous cells, scattered with raphides of calcium oxalate [Fig. 2 (ii)].

Powder

Colour greyish-brown. Cork cells polygonal to irregular, wall thick, containing brown contents. Raphides of calcium oxalate numerous, scattered singly or in bundles, 18-97 μm long; bright polychromatic under the polarized microscope. Fibres colourless, slender, slightly curved. Vessels scattered singly or in bundles, mostly bordered-pitted vessels and few spiral vessels, 4-84 μm in diameter. Tracheids numerous, mostly in bundles, wall slightly thick and pits clear (Fig. 3).

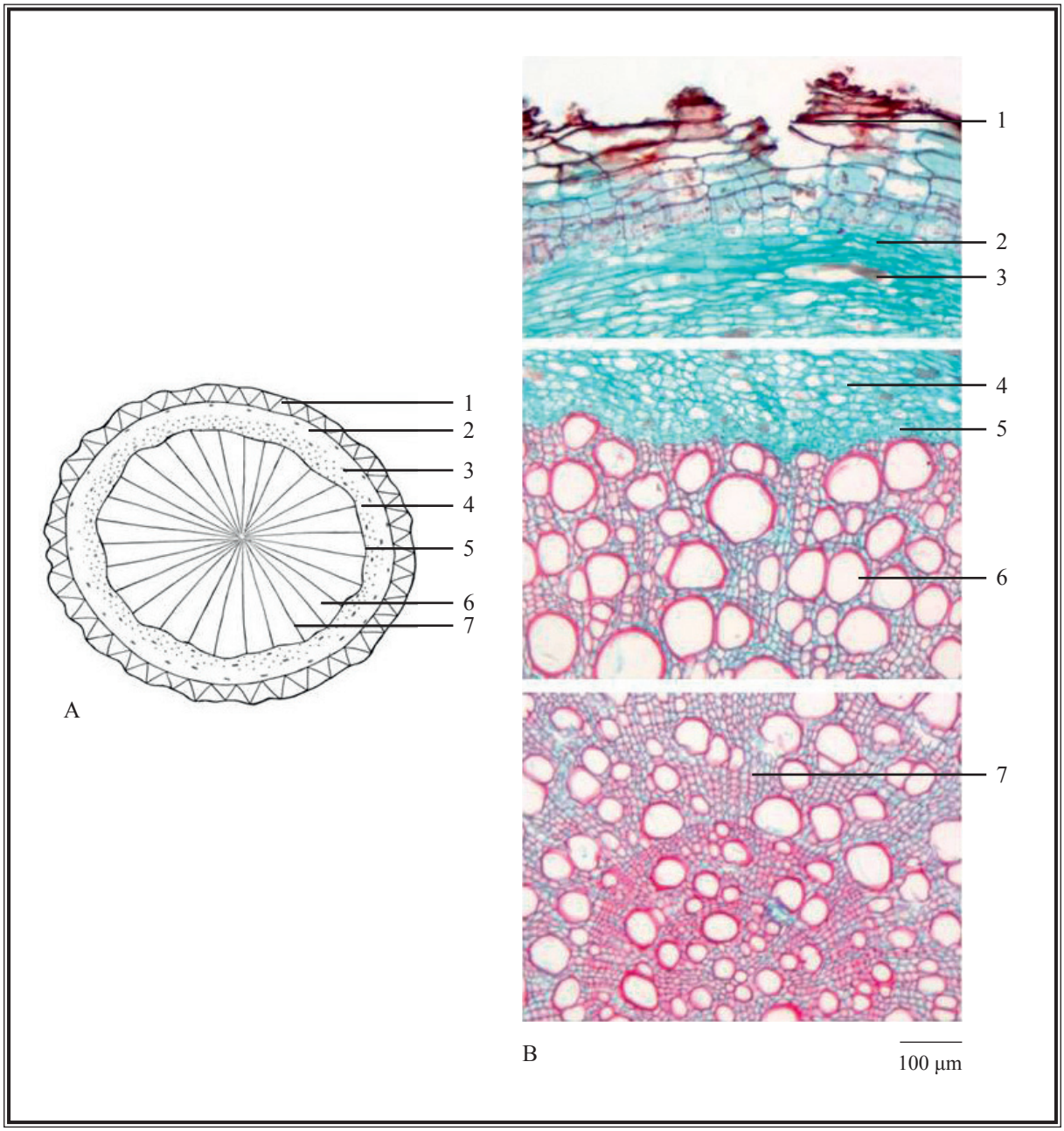

Figure 2 (i) Microscopic features of transverse section of root of Rubiae Radix et Rhizoma

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Raphides of calcium oxalate 4. Phloem 5. Cambium
6. Xylem 7. Xylem ray

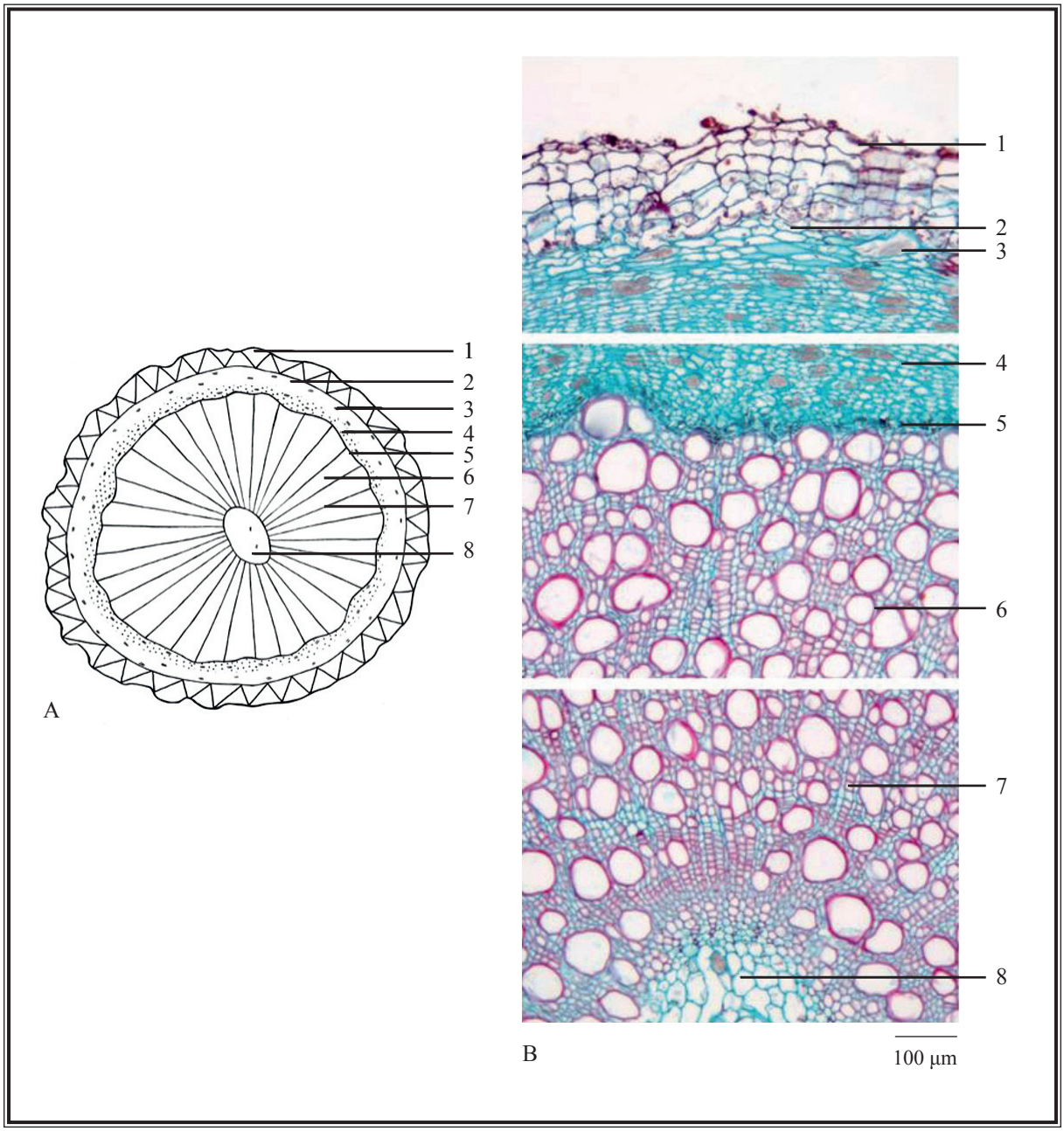

Figure 2 (ii) Microscopic features of transverse section of rhizome of Rubiae Radix et Rhizoma

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Raphides of calcium oxalate 4. Phloem 5. Cambium
6. Xylem 7. Xylem ray 8. Pith

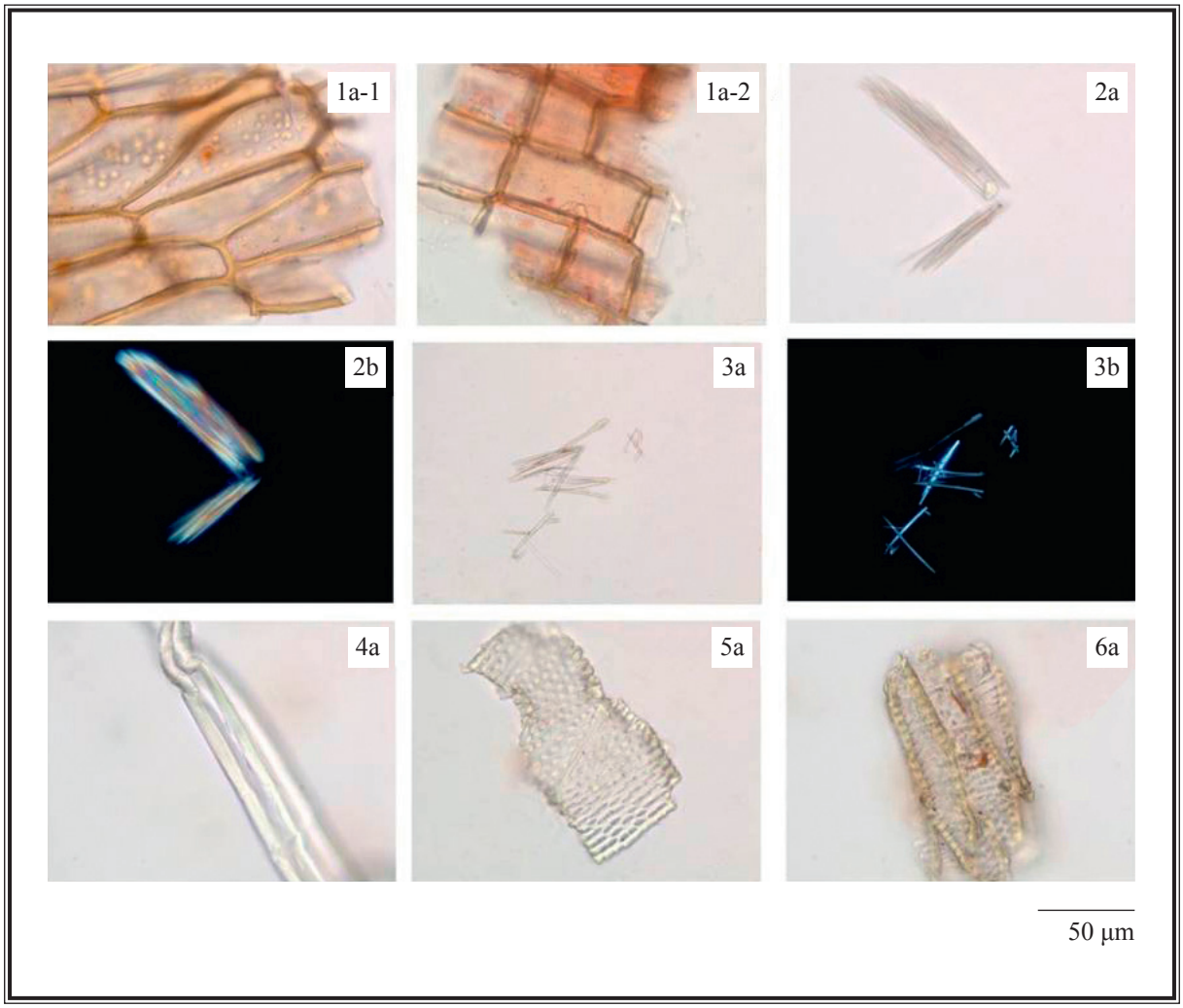

Figure 3 Microscopic features of powder of Rubiae Radix et Rhizoma

1. Cork cells 2. Raphides of calcium oxalate in bundles
3. Scattered raphides of calcium oxalate 4. Fibres 5. Bordered-pitted vessels
6. Tracheids

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

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

Standard solution

Mollugin standard solution

Weigh 2.5 mg of mollugin CRS (Fig. 4) and dissolve in 10 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate and formic acid (10:1:0.1, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (150 W) the mixture for 10 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 mollugin standard solution and the test solution (1 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. 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 mollugin.

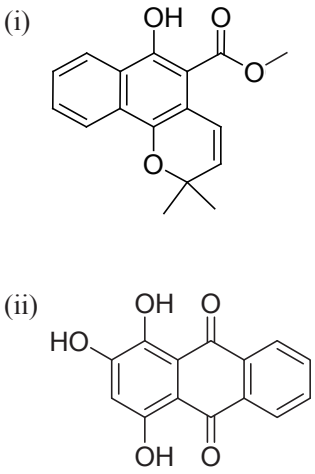

Figure 4 Chemical structures of (i) mollugin and (ii) purpurin

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

Standard solution

Mollugin standard solution for fingerprinting, Std-FP (12 mg/L)

Weigh 0.12 mg of mollugin CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL conical flask, then add 100 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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)	Acetonitrile (%, v/v)	0.2% Trifluoroacetic acid and 0.2% triethylamine (%, v/v)	Elution
0 – 60	20 → 100	80 → 0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of mollugin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of mollugin should not be more than 5.0%; the RSD of the retention time of mollugin peak should not be more than 2.0%; the column efficiency determined from mollugin peak should not be less than 200000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.15	± 0.03
2	0.19	± 0.03
3	0.39	± 0.03
4 (marker, mollugin)	1.00	-

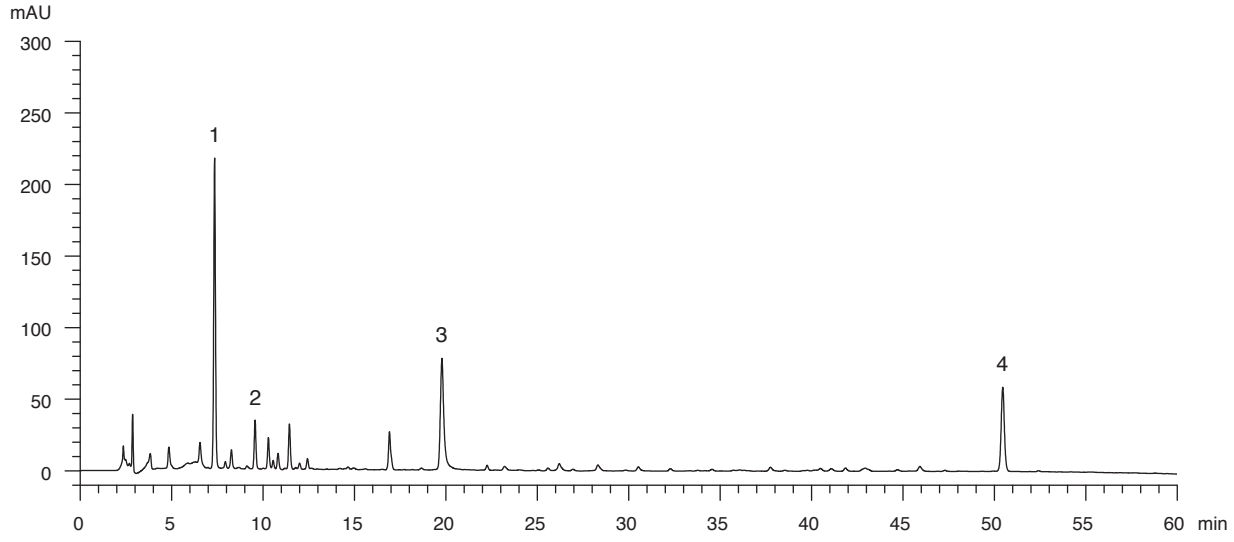

Figure 5 A reference fingerprint chromatogram of Rubiae Radix et Rhizoma extract

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. 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 15.0%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

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

7. ASSAY

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

Standard solution

Mixed mollugin and purpurin standard stock solution, Std-Stock (100 mg/L each)

Weigh accurately 1.0 mg of mollugin CRS and 1.0 mg of purpurin CRS (Fig. 5), and dissolve in 10 mL of ethanol (70%).

Mixed mollugin and purpurin standard solution for assay, Std-AS

Measure accurately the volume of the mixed mollugin and purpurin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 1, 2, 8, 16, 30 mg/L for both mollugin and purpurin.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 43 mL of ethanol (70%) and 2 mL of hydrochloric acid (81.6%, w/v). Reflux the mixture for 2 h. Cool down to room temperature. Add 2.25 mL triethylamine. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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)	Acetonitrile (%, v/v)	Methanol (%, v/v)	0.05% Phosphoric acid (%, v/v)	Elution
0 – 11	45	25	30	isocratic
11 – 30	45 → 75	25 → 20	30 → 5	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed mollugin and purpurin Std-AS (8 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of mollugin and purpurin should not be more than 5.0%; the RSD of the retention times of mollugin and purpurin peaks should not be more than 2.0%; the column efficiencies determined from mollugin and purpurin peaks should not be less than 10000 and 15000 theoretical plates respectively.

The *R* value between mollugin peak and the closest peak; and the *R* value between purpurin 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 mollugin and purpurin Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of mollugin and purpurin against the corresponding concentrations of the mixed mollugin and purpurin Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify mollugin and purpurin peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed mollugin and purpurin Std-AS. The retention times of mollugin and purpurin 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 mollugin and purpurin in the test solution, and calculate the percentage contents of mollugin and purpurin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.40% of mollugin ($C_{17}H_{16}O_4$) and not less than 0.42% of purpurin ($C_{14}H_8O_5$), calculated with reference to the dried substance.