Corydalis Decumbentis Rhizoma

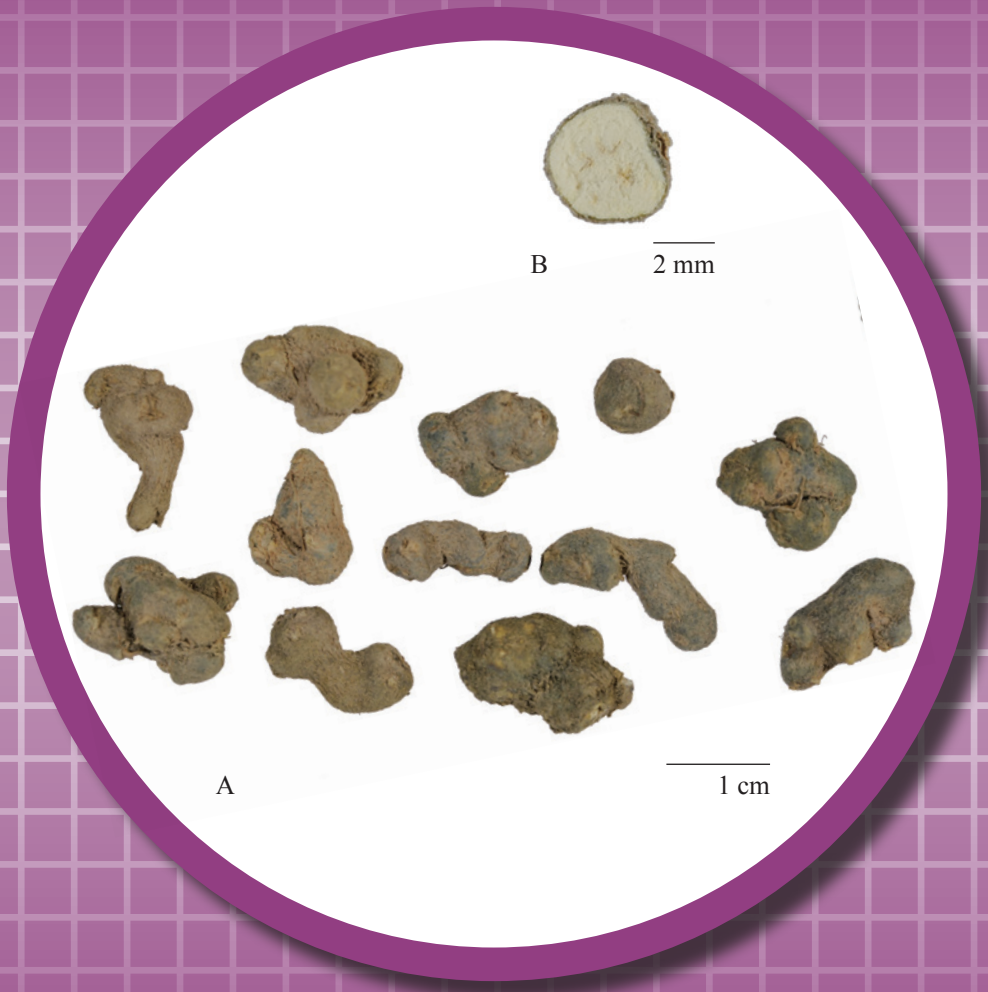

Figure 1 A photograph of Corydalis Decumbentis Rhizoma

A. Corydalis Decumbentis Rhizoma

B. Magnified image of transverse section of tuber

1. NAMES

Official Name: Corydalis Decumbentis Rhizoma

Chinese Name: 夏天無

Chinese Phonetic Name: Xiatianwu

2. SOURCE

Corydalis Decumbentis Rhizoma is the dried tuber of *Corydalis decumbens* (Thunb.) Pers. (Papaveraceae). The tuber is collected in spring or early summer after the seedlings have emerged, foreign matter removed, washed clean, and dried to obtain Corydalis Decumbentis Rhizoma.

3. DESCRIPTION

Subspheroidal, long-rounded or irregular, 0.4-3 cm long, 4-25 mm in diameter. Externally greyish-yellow, dark green or dark brown, with tubercular and indistinct fine wrinkles. Apex obtuse and rounded, stem scars visible, surrounded by numerous pale yellow dotted leaf scars and rootlet scars. Texture hard. Fracture yellowish-white or yellow, granular or corneous, some tubers slightly starchy. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of tangentially elongated cells. Cortex consists of several layers of flattened cells, the cells pale yellow, with pits. Vascular bundles collateral, 4-7, arranged radially. Phloem narrow; xylem ribbon-like, vessel minute. Pith situated in the centre (Fig. 2).

Powder

Colour pale yellowish-brown. Hypodermal cells in pieces, pale yellowish-brown, subrectangular or irregular in shape, walls slightly thickened in interrupted beaded shape, pits obvious. Starch granules faintly visible, single starch granules subrounded or oblong, hilum pointed or flyer-shaped; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-6 units. Vessels mainly reticulate, 9-43 μm in diameter (Fig. 3).

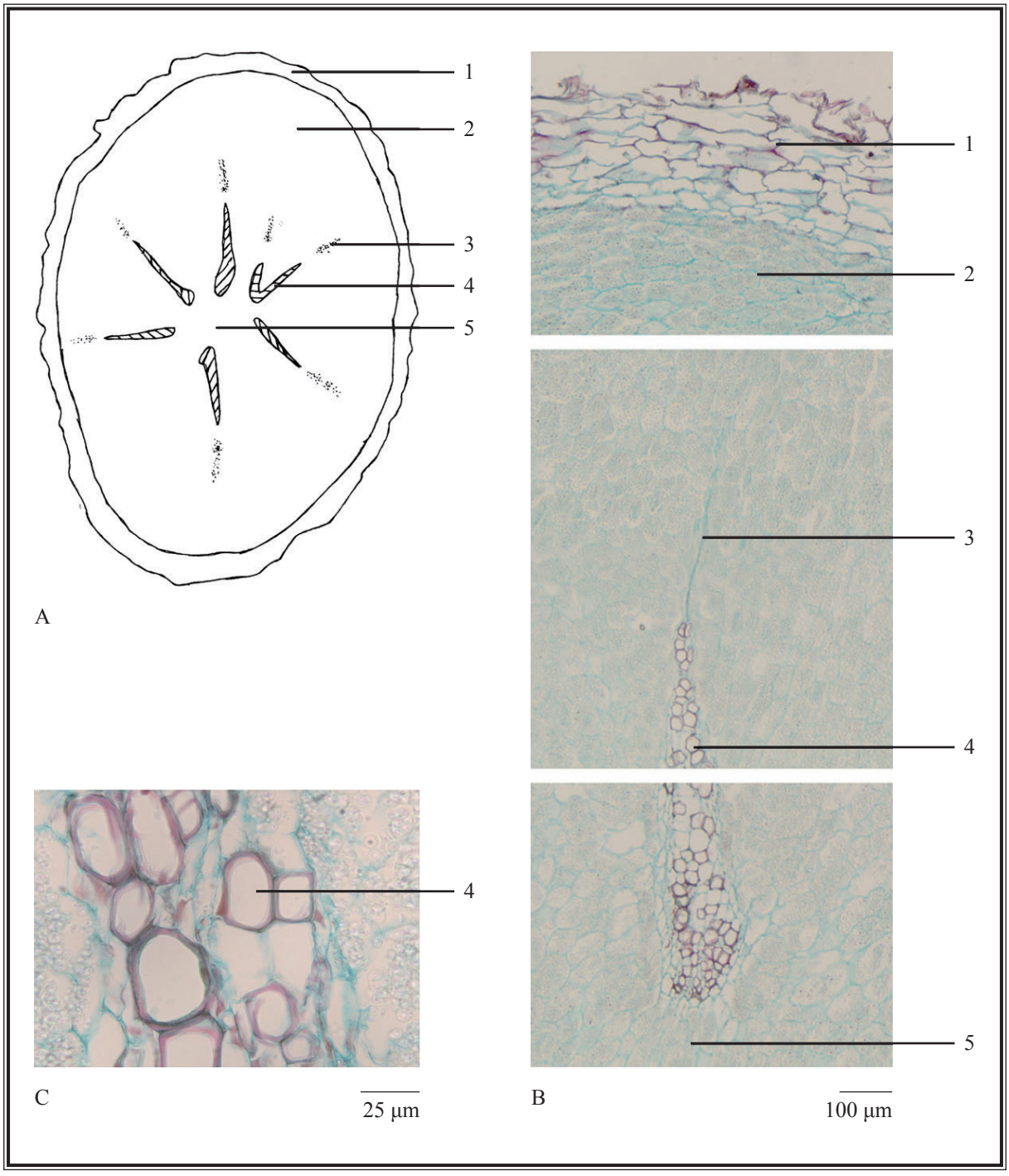

Figure 2 Microscopic features of transverse section of Corydalis Decumbentis Rhizoma

A. Sketch B. Section illustration C. Vessel

1. Cork 2. Cortex 3. Phloem 4. Xylem 5. Pith

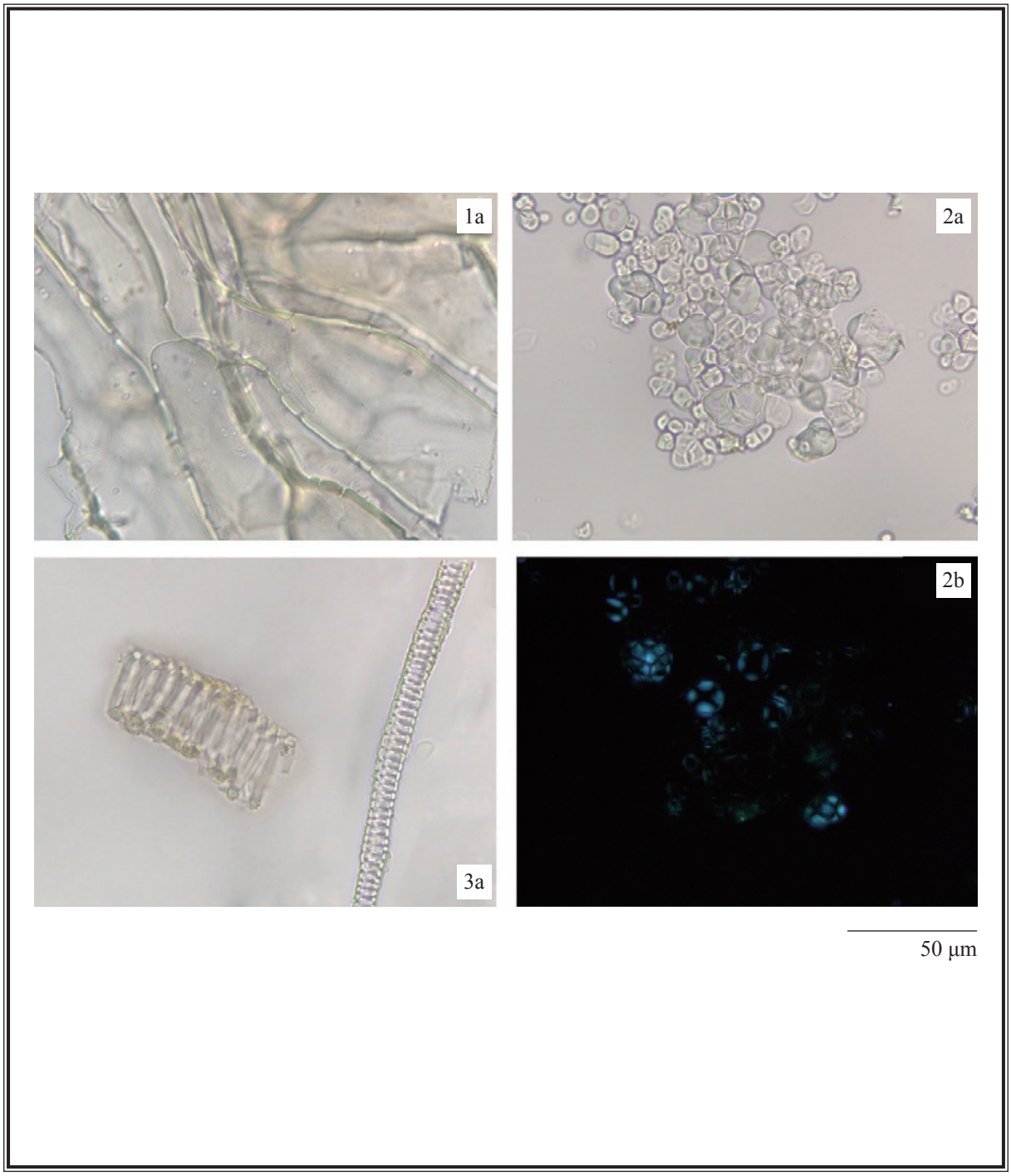

Figure 3 Microscopic features of powder of Corydalis Decumbentis Rhizoma

1. Hypodermal cells 2. Starch granules and gelatinized starch granules 3. Vessels

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

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

Standard solutions

Palmatine chloride standard solution

Weigh 1.0 mg of palmatine chloride CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Protopine standard solution

Weigh 0.5 mg of protopine CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of *n*-butanol, water and formic acid (7:2:1, v/v).

Staining reagent

Iodine.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 45 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 palmatine chloride standard solution (0.5 μL), protopine standard solution (2 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 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. Fumigate the plate with iodine vapor chamber until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

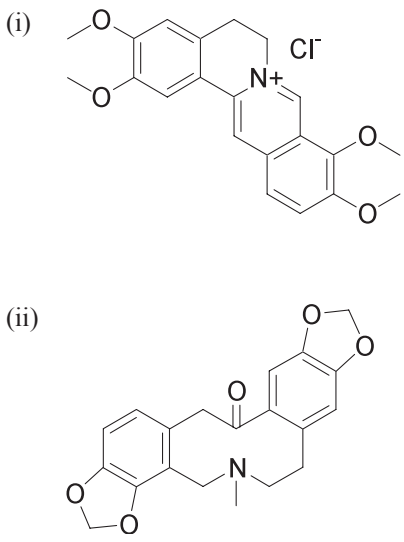

Figure 4 Chemical structures of (i) palmatine chloride and (ii) protopine

Figure 5 A reference HPTLC chromatogram of Corydalis Decumbentis Rhizoma extract observed under visible light after staining

1. Protopine standard solution 2. Palmatine chloride 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 palmatine and protopine (Fig. 5).

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

Standard solutions

Palmatine chloride standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of palmatine chloride CRS and dissolve in 10 mL of ethanol.

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

Weigh 0.5 mg of protopine CRS and dissolve in 10 mL of ethanol.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol (50%). Sonicate (150 W) the mixture for 1 h. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). 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)	0.06% Trifluoroacetic acid and 0.1% triethylamine (%, v/v)	Elution
0 – 20	7 → 17	93 → 83	linear gradient
20 – 60	17 → 25	83 → 75	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of palmatine chloride Std-FP and protopine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of palmatine and protopine should not be more than 5.0%; the RSD of the retention times of palmatine and protopine peaks should not be more than 2.0%; the column efficiencies determined from palmatine and protopine peaks should not be less than 70000 and 50000 theoretical plates respectively.

The *R* value between peak 4 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject palmatine chloride Std-FP, protopine Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of palmatine and protopine peaks in the chromatograms of palmatine chloride Std-FP, protopine Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify palmatine and protopine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of palmatine chloride Std-FP and protopine Std-FP. The retention times of palmatine and protopine 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 five characteristic peaks of Corydalis Decumbentis Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Corydalis Decumbentis Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.33	± 0.03
2	0.49	± 0.03
3	0.80	± 0.03
4 (marker, protopine)	1.00	-
5 (palmatine)	1.46	± 0.03

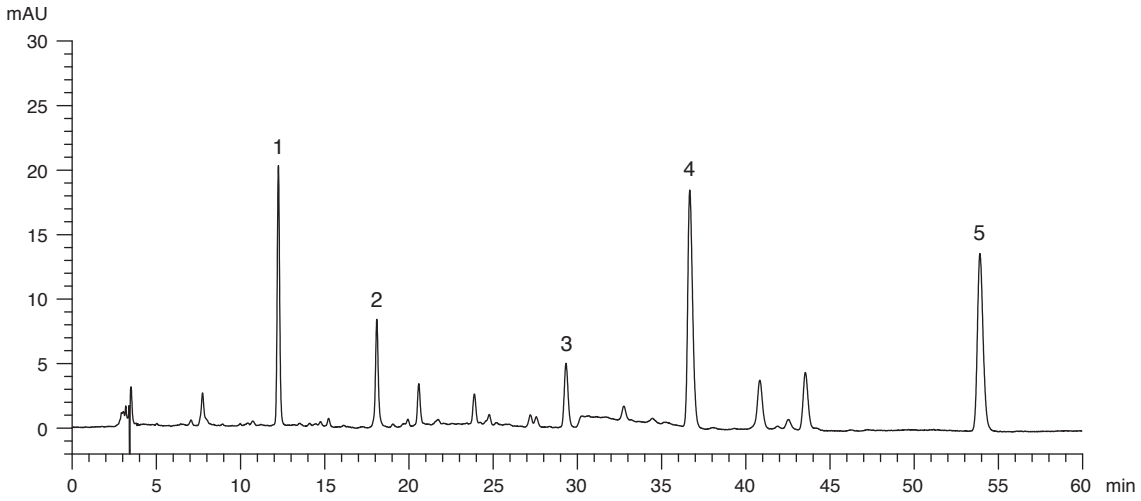

Figure 6 A reference fingerprint chromatogram of Corydalis Decumbentis Rhizoma extract

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. 6).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins – Aflatoxins *(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 5.0%.
Acid-insoluble ash: not more than 5.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 28.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

Mixed palmatine chloride and protopine standard stock solution, Std-Stock (100 mg/L for each)

Weigh accurately 1.0 mg of palmatine chloride CRS and 1.0 mg of protopine CRS, and dissolve in 10 mL of ethanol.

Mixed palmatine chloride and protopine standard solution for assay, Std-AS

Measure accurately the volume of the mixed palmatine chloride and protopine Std-Stock, dilute with ethanol to produce a series of solutions of 5, 10, 15, 30, 60 mg/L for palmatine chloride and 5, 10, 25, 40, 80 mg/L for protopine.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (290 nm for protopine and 345 nm for palmatine) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). 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)	0.06% Trifluoroacetic acid and 0.1% triethylamine (%, v/v)	Elution
0 – 10	22 → 25	78 → 75	linear gradient
10 – 25	25 → 29	75 → 71	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed palmatine chloride and protopine Std-AS (15 mg/L for palmatine chloride and 25 mg/L for protopine). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of palmatine and protopine should not be more than 5.0%; the RSD of the retention times of palmatine and protopine peaks should not be more than 2.0%; the column efficiencies determined from palmatine and protopine peaks should not be less than 15000 and 10000 theoretical plates respectively.

The *R* value between palmatine peak and the closest peak; and the *R* value between protopine 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 palmatine chloride and protopine Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of palmatine and protopine against the

corresponding concentrations of the mixed palmatine chloride and protopine 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 palmatine and protopine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed palmatine chloride and protopine Std-AS. The retention times of palmatine and protopine 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 palmatine and protopine in the test solution, and calculate the percentage contents of palmatine (the percentage content of palmatine chloride × 0.91, where 0.91 is the molar mass ratio of palmatine and palmatine chloride) and protopine in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.15% of palmatine ($C_{21}H_{22}NO_4$) and not less than 0.32% of protopine ($C_{20}H_{19}NO_5$), calculated with reference to the dried substance.