Picrorhizae Rhizoma

Figure 1 A photograph of Picrorhizae Rhizoma

A. Picrorhizae Rhizoma B. Magnified transverse section of rhizome

1. NAMES

Official Name: Picrorhizae Rhizoma

Chinese Name: 胡黃連

Chinese Phonetic Name: Huhuanglian

2. SOURCE

Picrorhizae Rhizoma is the dried rhizome of *Picrorhiza scrophulariiflora* Pennell (Scrophulariaceae). The rhizome is collected in autumn, fibrous root and soil removed, then dried under the sun to obtain Picrorhizae Rhizoma.

3. DESCRIPTION

Cylindrical, slightly curved, occasionally branched, 3-12 cm long, 3-10 mm in diameter. Externally greyish-brown to dark brown, rough, with dense annulate nodes, exhibiting slightly protuberant scars of buds and fibrous roots, closely covered with dark brown scaly remnants of petiole at the upper part. Texture hard, fragile and light in weight, easily broken. Fracture slightly even, pale brown to dark brown, wood exhibiting 4-10 whitish dotted vascular bundles arranged in a ring. Odour slight; taste extremely bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers to more than 10 layers of cells. Cortex cells oblong or rectangle, tangentially prolonged. Endodermis cells rectangle. Phloem consists of 9-13 layers of polygonal or oblong cells. Xylem ray varying in rows and up to more than 9 rows of cells. Xylem vessels mostly in groups. Pith cells subrounded to polygonal (Fig. 2).

Powder

Colour brown. Cork cells yellowish-brown, polygonal to irregular in surface view, rectangular in lateral view. Parenchymatous cells long-ovoid or irregular, cell walls thickened or beaded-thickened, pits visible. Vessels mostly reticulate, 8-28 μm in diameter (Fig. 3).

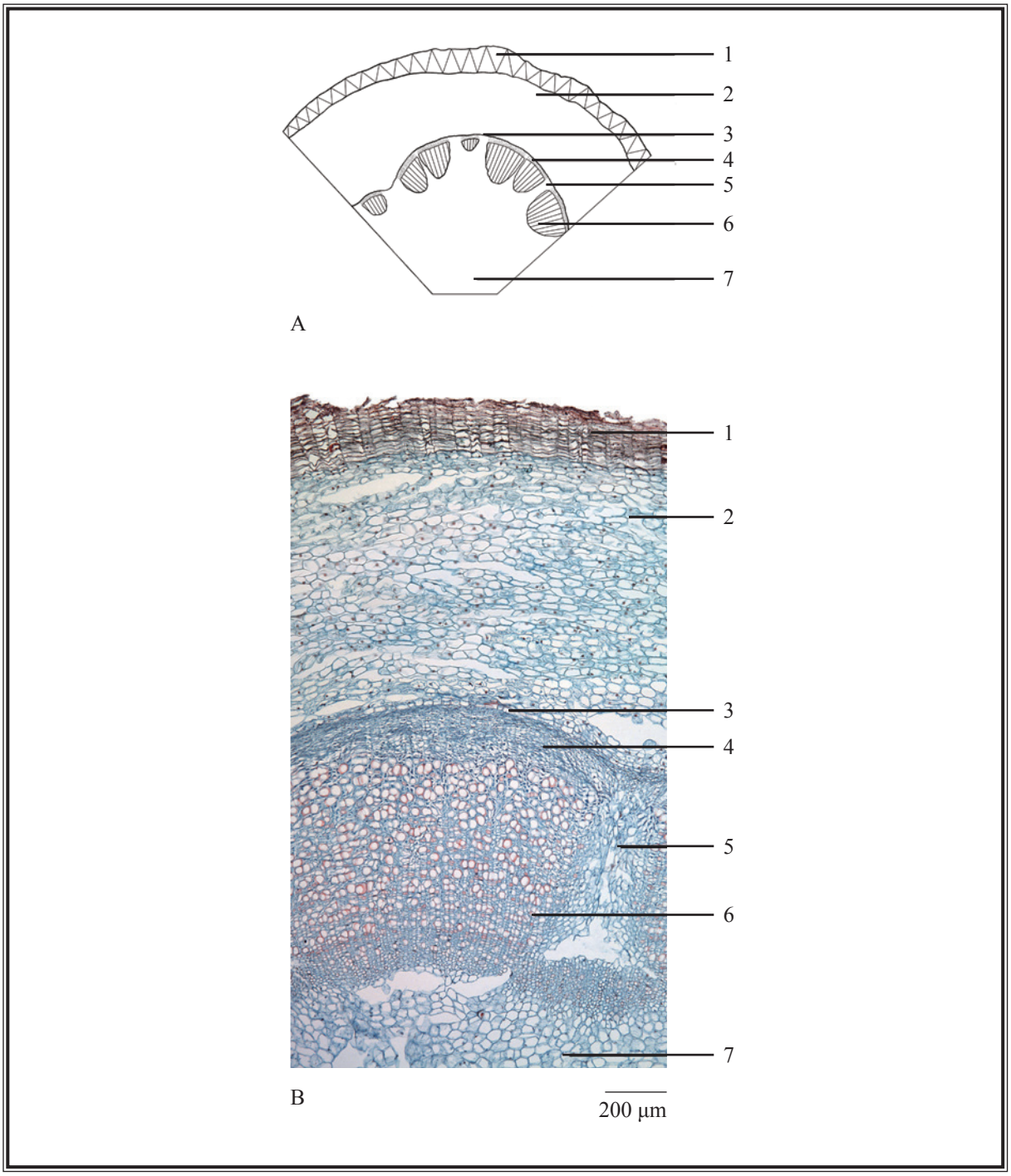

Figure 2 Microscopic features of transverse section of Picrorhizae Rhizoma

A. Sketch B. Section illustration

1. Cork 2. Cortex 3. Endodermis 4. Phloem 5. Xylem ray 6. Xylem 7. Pith

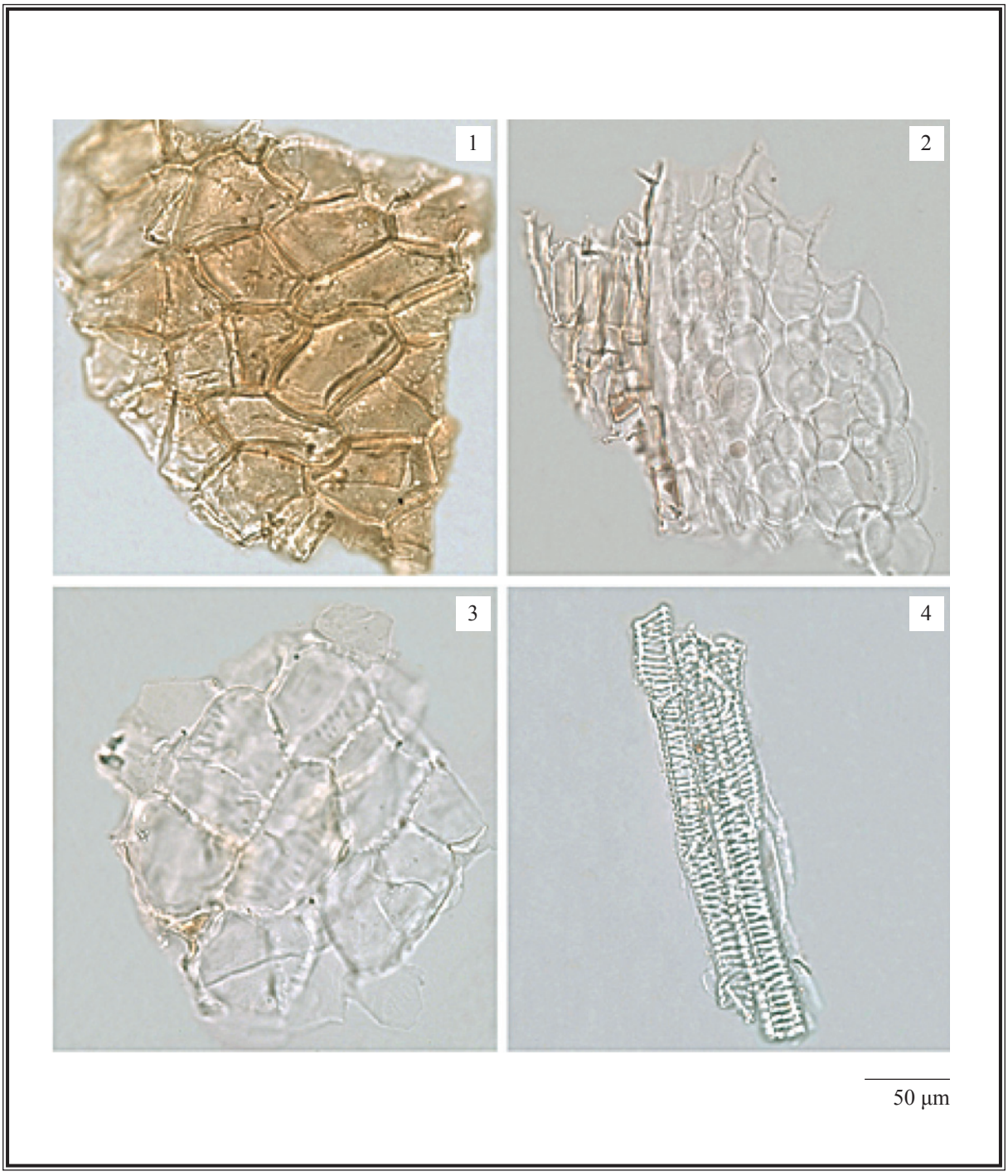

Figure 3 Microscopic features of powder of Picrorhizae Rhizoma (under the light microscope)

1. Cork cells 2. Cork cells and adjacent parenchymatous cells
3. Parenchymatous cells with beaded-thickened walls 4. Vessels

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

Standard solutions

Picroside I standard solution

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

Picroside II standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and glacial acetic acid (6:1:0.2, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (270 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 picroside I standard solution, picroside II 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 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

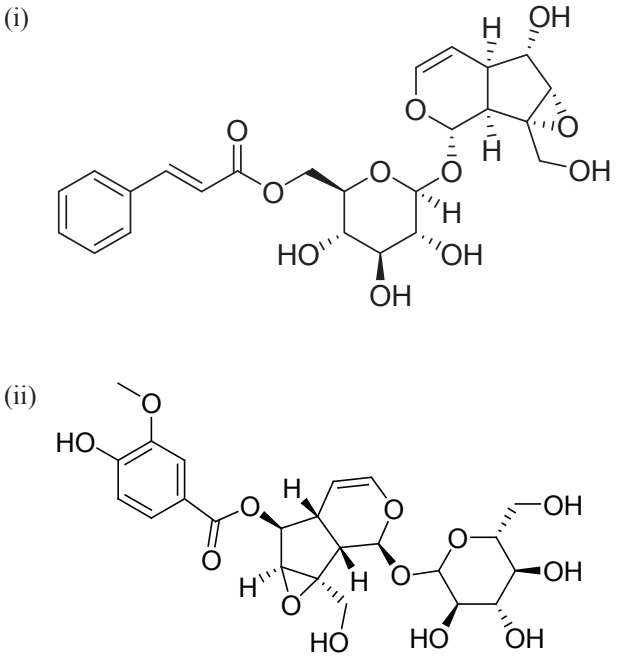

Figure 4 Chemical structures of (i) picroside I and (ii) picroside II

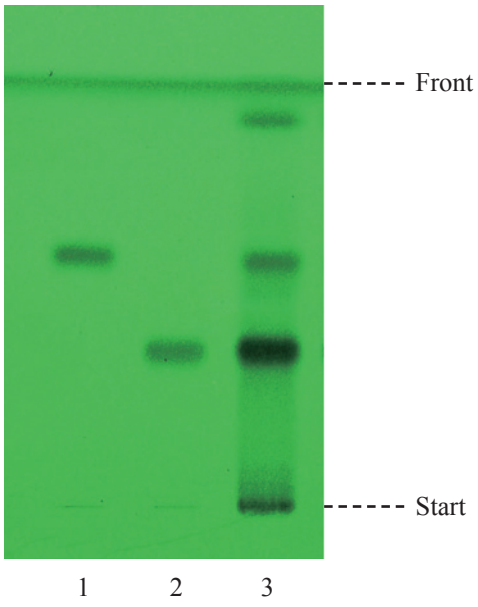

Figure 5 A reference HPTLC chromatogram of Picrorhizae Rhizoma extract observed under UV light (254 nm)

1. Picroside I standard solution 2. Picroside II 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 picroside I and picroside II (Fig. 5).

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

Standard solutions

Picroside I standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of picroside I CRS and dissolve in 10 mL of water.

Picroside II standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of picroside II CRS and dissolve in 10 mL of water.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 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)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of picroside I Std-FP and picroside II Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of picroside I and picroside II should not be more than 5.0%; the RSD of the retention times of picroside I and picroside II peaks should not be more than 2.0%; the column efficiencies determined from picroside I and picroside II peaks should not be less than 100000 and 60000 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 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Picrorhizae Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.41	± 0.03
2	0.57	± 0.03
3 (marker, picroside II)	1.00	-
4 (picroside I)	1.28	± 0.03

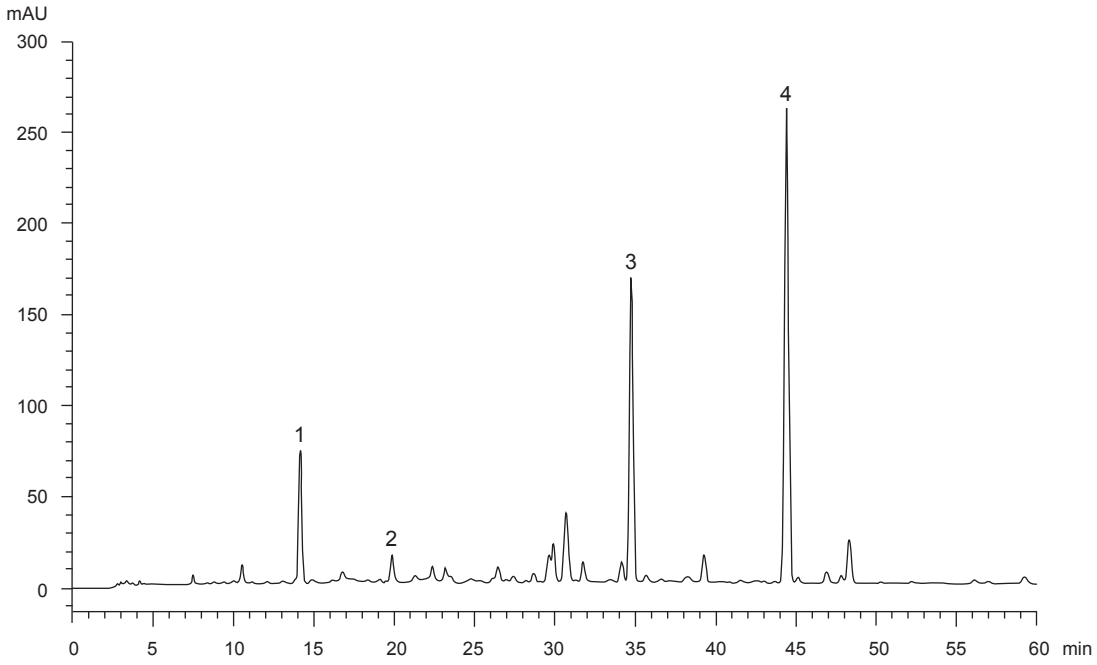

Figure 6 A reference fingerprint chromatogram of Picrorhizae Rhizoma extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 28.0%.

7. ASSAY

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

Standard solution

Mixed picroside I and picroside II standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 5.0 mg of picroside I CRS and 5.0 mg of picroside II CRS, and dissolve in 5 mL of water.

Mixed picroside I and picroside II standard solution for assay, Std-AS

Measure accurately the volume of the mixed picroside I and picroside II Std-Stock, dilute with water to produce a series of solutions of 2.5, 50, 200, 300, 400 mg/L for both picroside I and picroside II.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (275 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)	0.4% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed picroside I and picroside II Std-AS (200 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of picroside I and picroside II should not be more than 5.0%; the RSD of the retention times of picroside I and picroside II peaks should not be more than 2.0%; the column efficiencies determined from picroside I and picroside II peaks should not be less than 100000 and 60000 theoretical plates respectively.

The *R* value between picroside I peak and the closest peak; and the *R* value between picroside II 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 picroside I and picroside II Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of picroside I and picroside II against the corresponding concentrations of the mixed picroside I and picroside II 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 picroside I and picroside II peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed picroside I and picroside II Std-AS. The retention times of picroside I and picroside II 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 picroside I and picroside II in the test solution, and calculate the percentage contents of picroside I and picroside II in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 4.5% of the total content of picroside I ($C_{24}H_{28}O_{11}$) and picroside II ($C_{23}H_{28}O_{13}$), calculated with reference to the dried substance.