Peucedani Radix

Figure 1 A photograph of Peucedani Radix

1. NAMES

Official Name: Peucedani Radix

Chinese Name: 前胡

Chinese Phonetic Name: Qianhu

2. SOURCE

Peucedani Radix is the dried root of *Peucedanum praeruptorum* Dunn (Apiaceae). The root is collected in winter to next spring, when stem and leaves wither or before floral stem grows, freed of aerial part, soil and rootlet, washed clean, then dried under the sun or at ambient temperature to obtain Peucedani Radix.

3. DESCRIPTION

Irregular cylindrical, conical or fusiform, slightly twisted, frequently branched at the lower part, 4-15 cm long, 3-18 mm in diameter. Externally yellowish-brown to dark brown, marked with longitudinal furrows or wrinkles and transverse lenticel-like cicatrices. The top part is stubby, frequently with stem scars and fibrous leaf-bases remains at the root stock, with numerous fine annular striations at the upper end. Texture relatively flexible, and hard when dried, easily broken; fracture uneven, pale yellow, numerous brownish-yellow oil spots scattered in cortex, cambium ring brown. Odour aromatic; taste slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 10 or more layers of flatted cells. Cortex narrow. Phloem broad, cells at the outside frequently with clefts, oil cavities frequently scattered, 40-330 μm in diameter. Phloem ray slightly curve. Cambium in a ring. Xylem vessels scattered singly or aggregated in groups, radially arranged, xylem rays distinct, with oil cavities scattered in the xylem. Parenchymatous cells contain abundant starch granules (Fig. 2).

Powder

Colour greyish-yellow to yellowish-brown. Starch granules abundant, simple granules

subrounded or polygonal, 3-25 μm in diameter, dotted or slit-shaped hilum visible in large granules; compound granules composed of 2-10 units; black and cruciate in shape under the polarized microscope. Fibres in bundles or scattered singly, 86-300 μm long, 11-40 μm in diameter. Pit sparse, pit canals occasionally visible; yellowish-white under the polarized microscope. Stone cells singly scattered, subrectangular or elongated ovate, 33-152 μm long, 9-60 μm in diameter, the wall thick, striation mostly distinct; yellowish-white under the polarized microscope. Vessels mainly reticulate, 12-79 μm in diameter. Oil cavities mostly fractured, containing yellowish-brown or yellowish-green secretions or oil droplets. Cork cells yellowish-brown, subpolygonal, rectangular or triangular (Fig. 3).

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

Standard solutions

Praeruptorin A standard solution

Weigh 1.0 mg of praeruptorin A CRS (Fig. 4) and dissolve in 1 mL of methanol.

Praeruptorin B standard solution

Weigh 1.0 mg of praeruptorin B CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane and acetone (10:0.5, 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 methanol. Sonicate (90 W) the mixture for 15 min. Filter the mixture.

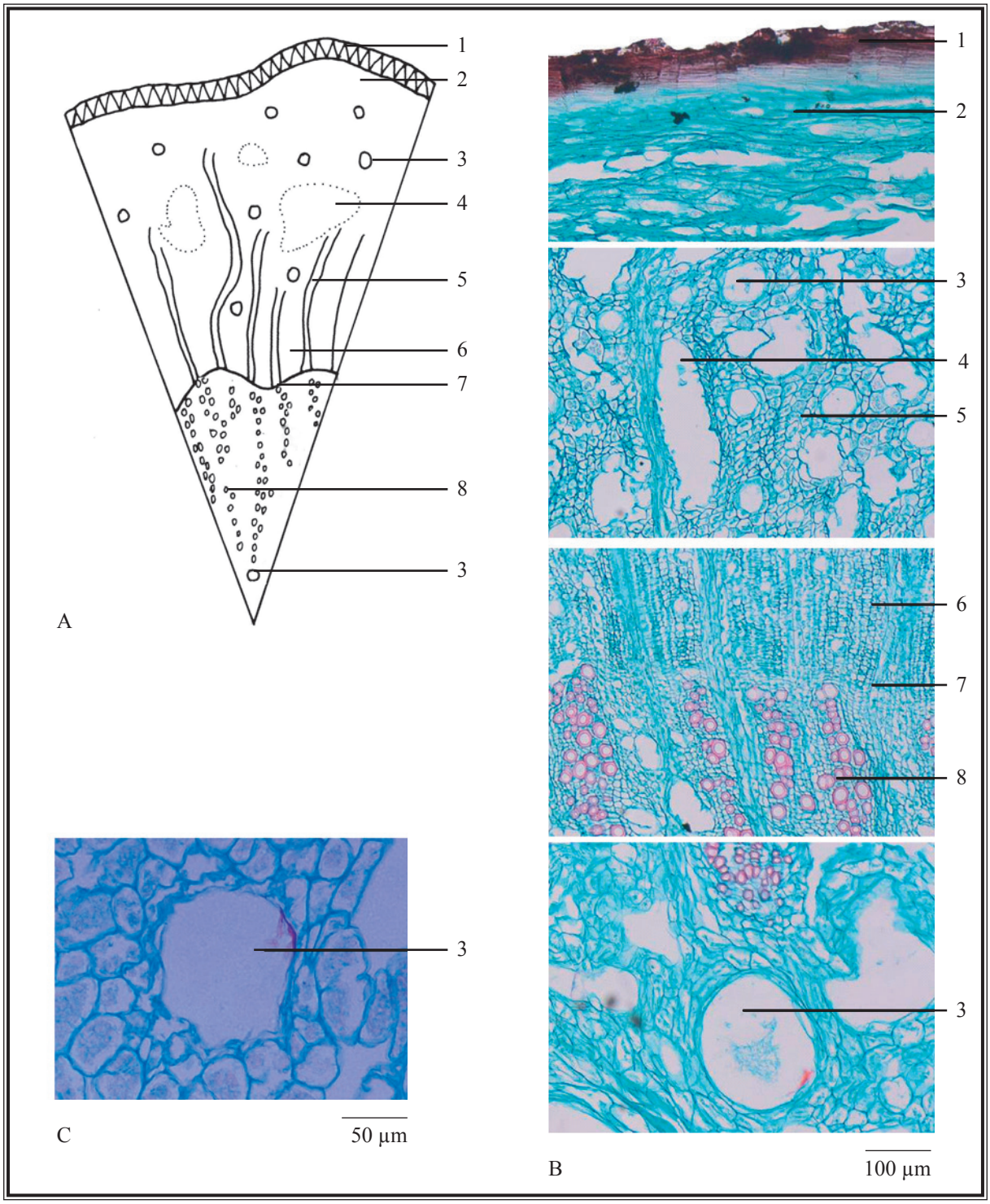


Figure 2 Microscopic features of transverse section of Peucedani Radix

A. Sketch B. Section illustration C. Oil cavity

1. Cork 2. Cortex 3. Oil cavity 4. Cleft 5. Phloem ray 6. Phloem 7. Cambium 8. Xylem

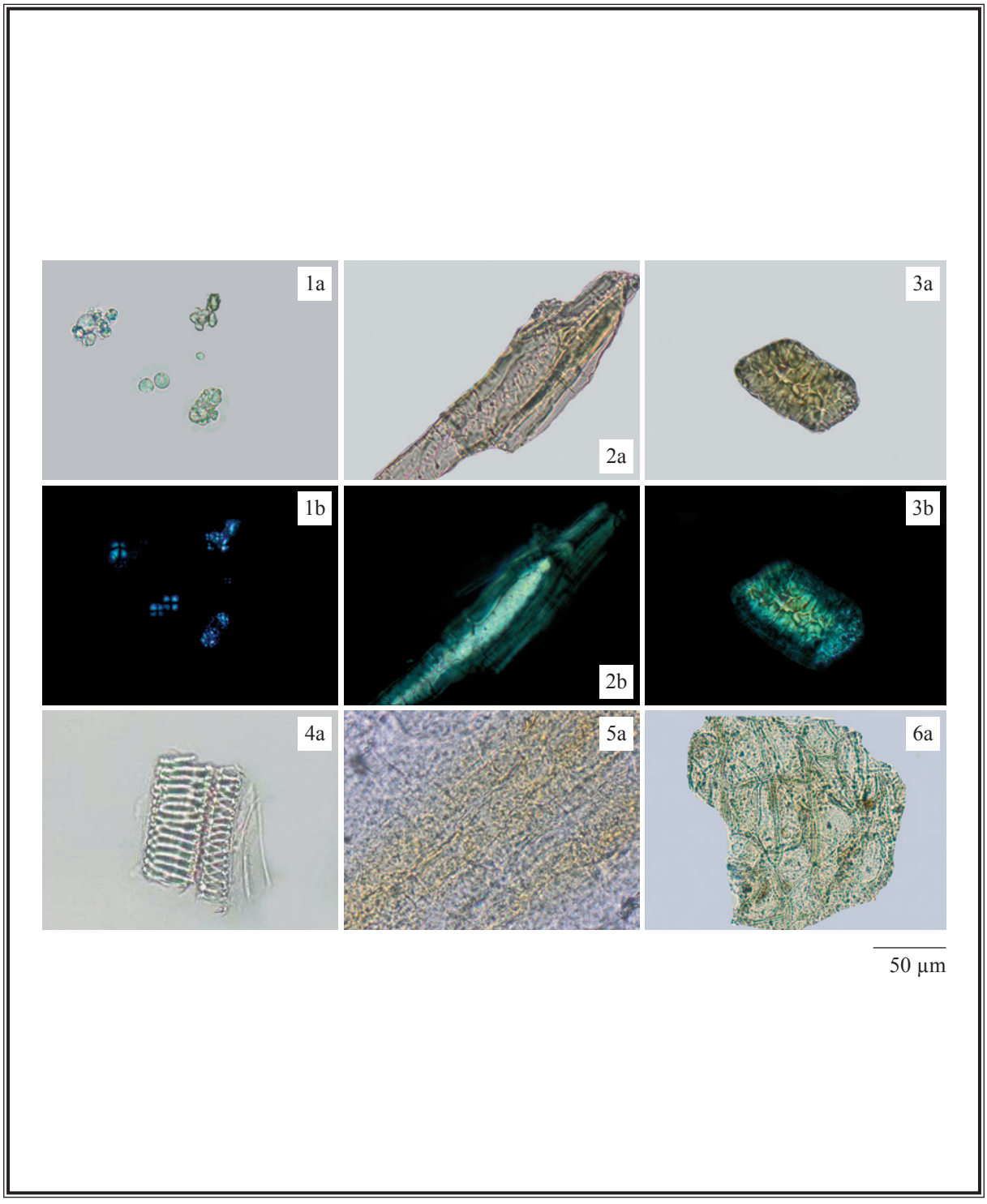
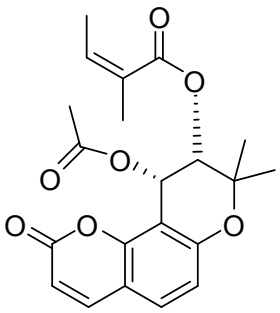


Figure 3 Microscopic features of powder of Peucedani Radix

1. Starch granules 2. Fibre 3. Stone cell 4. Vessels 5. Fragments of oil cavities 6. Cork cells

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

(i)



(ii)

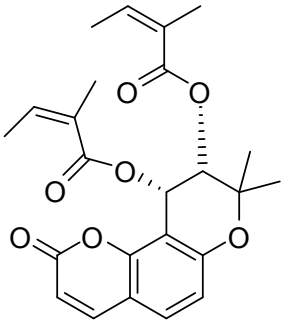


Figure 4 Chemical structures of (i) praeruptorin A and (ii) praeruptorin B

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 praeruptorin A standard solution, praeruptorin B 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 8.5 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).

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 praeruptorin A and praeruptorin B.

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

Standard solutions

Praeruptorin A standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of praeruptorin A CRS and dissolve in 10 mL of methanol.

Praeruptorin B standard solution for fingerprinting, Std-FP (6 mg/L)

Weigh 0.3 mg of praeruptorin B CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 125-mL conical flask, then add 50 mL of methanol. Sonicate (90 W) the mixture for 30 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (325 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 30	25 → 5	75 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of praeruptorin A Std-FP and praeruptorin B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of praeruptorin A and praeruptorin B should not be more than 5.0%; the RSD of the retention times of praeruptorin A and praeruptorin B peaks should not be more than 2.0%; the column efficiencies determined from praeruptorin A and paeruptorin B peaks should not be less than 9000 and 11000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Peucedani Radix extract

Peak No.	RRT	Acceptable Range
1 (praeruptorin A)	0.67	± 0.03
2	0.74	± 0.03
3 (marker, praeruptorin B)	1.00	-
4 (praeruptorin E and qianhucoumarin H)	1.16	± 0.03

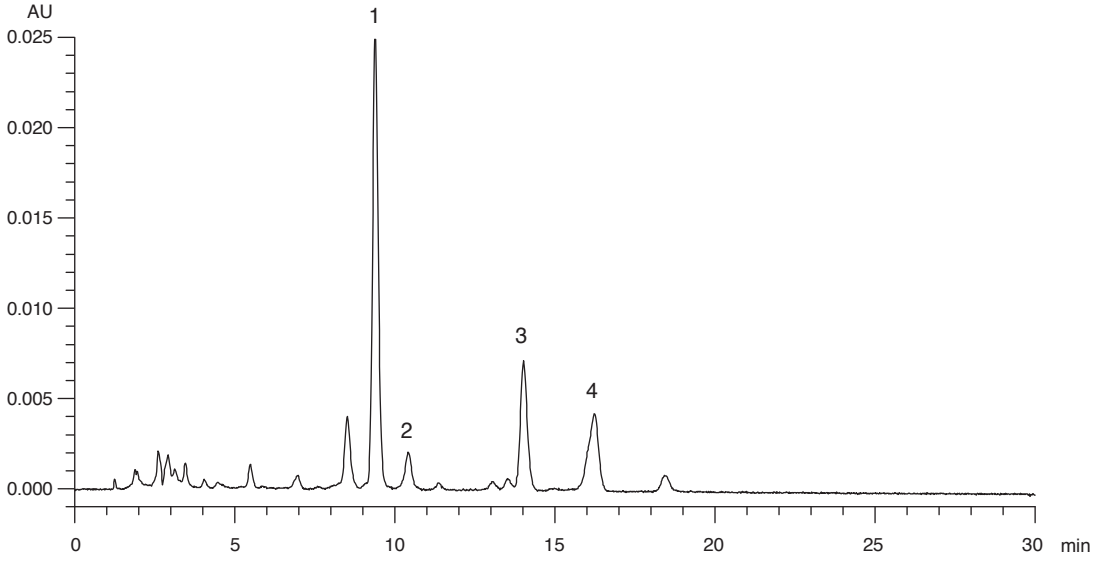


Figure 5 A reference fingerprint chromatogram of Peucedani Radix extract

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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.

Acid-insoluble ash: not more than 1.0%.

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 24.0%.

Ethanol-soluble extractives (hot extraction method): not less than 26.0%.

7. ASSAY

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

Standard solution

Mixed praeruptorin A and praeruptorin B standard stock solution, Std-Stock (400 mg/L for praeruptorin A and 120 mg/L for praeruptorin B)

Weigh accurately 2.0 mg of praeruptorin A CRS and 0.6 mg of praeruptorin B CRS, and dissolve in 5 mL of methanol.

Mixed praeruptorin A and praeruptorin B standard solution for assay, Std-AS

Measure accurately the volume of the mixed praeruptorin A and praeruptorin B Std-Stock, dilute with methanol to produce a series of solutions of 5, 10, 20, 40, 60 mg/L for praeruptorin A and 1.5, 3, 6, 12, 18 mg/L for praeruptorin B.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 125-mL conical flask, then add 50 mL of methanol. Sonicate (90 W) the mixture for 30 min. Transfer the solution to a 50-mL volumetric flask. Make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (325 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 30	25 → 5	75 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed praeruptorin A and praeruptorin B Std-AS (20 mg/L for praeruptorin A and 6 mg/L for praeruptorin B). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of praeruptorin A and praeruptorin B should not be more than 5.0%; the RSD of the retention times of praeruptorin A and praeruptorin B peaks should not be more than 2.0%; the column efficiencies determined from praeruptorin A and paeruptorin B peaks should not be less than 9000 and 11000 theoretical plates respectively.

The *R* value between praeruptorin A peak and the closest peak; and the *R* value between praeruptorin B 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 praeruptorin A and praeruptorin B Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of praeruptorin A and praeruptorin B against the corresponding concentrations of the mixed praeruptorin A and praeruptorin B 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 praeruptorin A and praeruptorin B peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed praeruptorin A and praeruptorin B Std-AS. The retention times of praeruptorin A and praeruptorin B peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of praeruptorin A and praeruptorin B in the test solution, and calculate the percentage contents of praeruptorin A and praeruptorin B in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.90% of praeruptorin A ($C_{21}H_{22}O_7$) and not less than 0.24% of praeruptorin B ($C_{24}H_{26}O_7$), calculated with reference to the dried substance.