Rhodiolae Crenulatae Radix et Rhizoma

Figure 1 A photograph of Rhodiolae Crenulatae Radix et Rhizoma

A. Rhodiolae Crenulatae Radix et Rhizoma B, C. Transverse section of rhizome

1. NAMES

Official Name: Rhodiolae Crenulatae Radix et Rhizoma

Chinese Name: 紅景天

Chinese Phonetic Name: Hongjingtian

2. SOURCE

Rhodiolae Crenulatae Radix et Rhizoma is the dried root and rhizome of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba (Crassulaceae). The root and rhizome is collected in autumn after scape withered, coarse bark removed, then dried under the sun to obtain Rhodiolae Crenulatae Radix et Rhizoma.

3. DESCRIPTION

Mostly are rhizome, root rarely found. Rhizome cylindrical or cut into irregular pieces, varying in length and up to 22 cm long, 70 mm in diameter. Externally reddish-brown, brown to dark brown, rough, with folds. The surface showing yellow membranous epidermis and pink stripe after peeling off the outer coarse bark. Internode irregular. Texture loose and light in weight, fracture orange-red to purplish-red, with an annulation. Odour aromatic; taste slightly bitter and astringent, then sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Rhizome: Cork consists of several to more than 30 layers of cells. Cortex cells elliptic or subrounded, varying in size. Sometimes parenchymatous cells contain brown secretions. Collateral vascular bundles radially, arranged in an interrupted ring. Fascicular cambium distinct. Xylem mostly present in inverted conical shape. Anomalous vascular bundles in pith, irregularly arranged into 1 to several layers, phloem of the outermost vascular bundles toward inside arranged, and further inward in the pith, vascular bundles usually appearing 3 forms: several rings with subrounded or subelliptic shape, each ring contains 2-10 collateral vascular bundles; collateral vascular bundles orientation varying; amphicribral. Pith broad, occupying up to 3/5 of the rhizome. Parenchymatous cells subrectangle, subround or irregular in shape (Fig. 2).

Powder

Colour brick-red. Vessels mostly reticulate. Cork cells brick-red, brownish-red or pale brown, polygonal to long polygonal in surface view. Parenchymatous cells contain yellowish-brown secretions, secretion masses irregular in shape, varied in size. Starch granules mostly simple, suborbicular or oval, 3-18 μm in diameter; black and cruciate-shaped under the polarized microscope (Fig. 3).

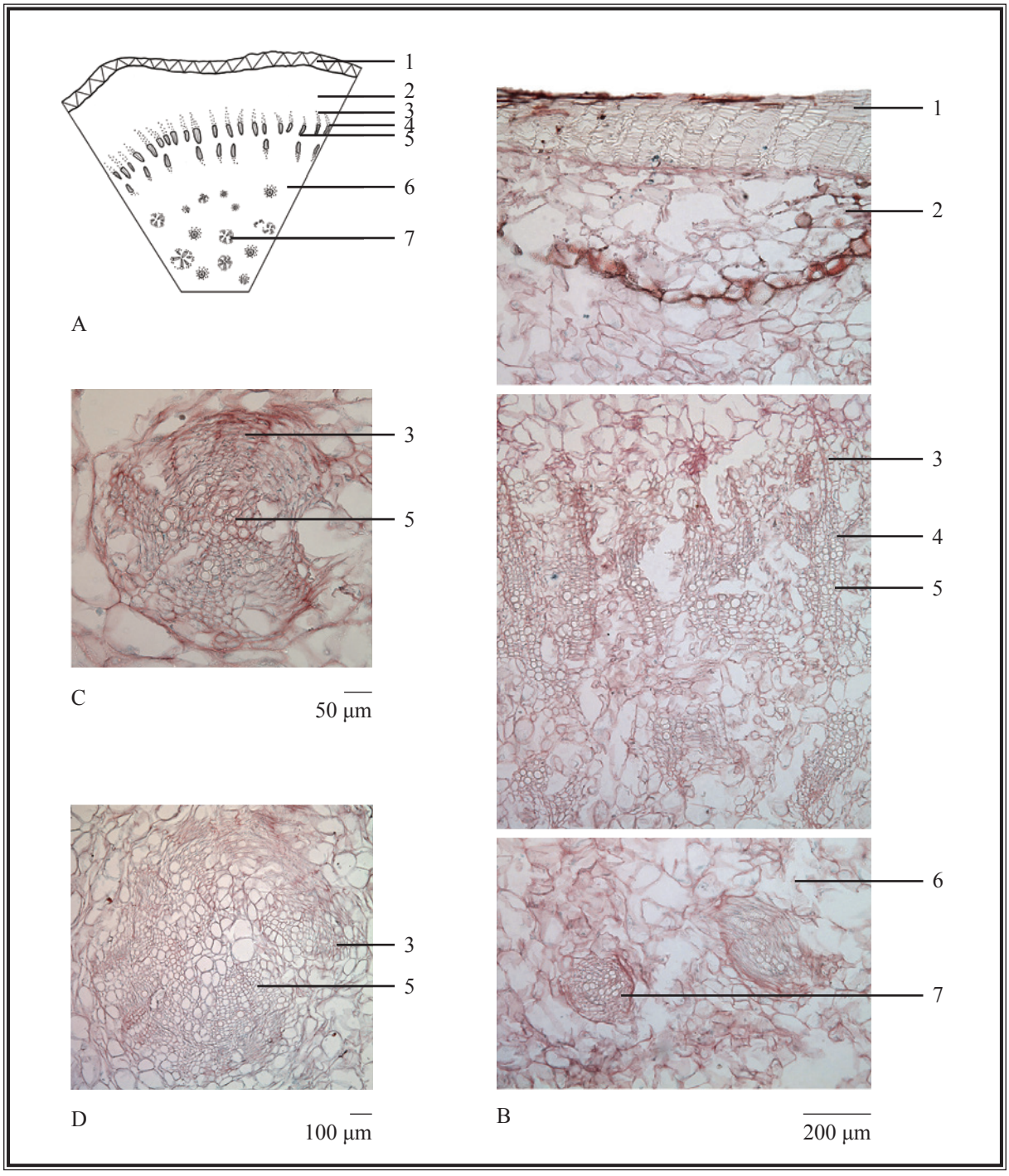

Figure 2 Microscopic features of transverse section of rhizome of Rhodiolae Crenulatae Radix et Rhizoma

A. Sketch B. Section illustration C. Amphicribral anomalous vascular bundle
D. Collateral anomalous vascular bundles

1. Cork 2. Cortex 3. Phloem 4. Fascicular cambium
5. Xylem 6. Pith 7. Anomalous vascular bundles

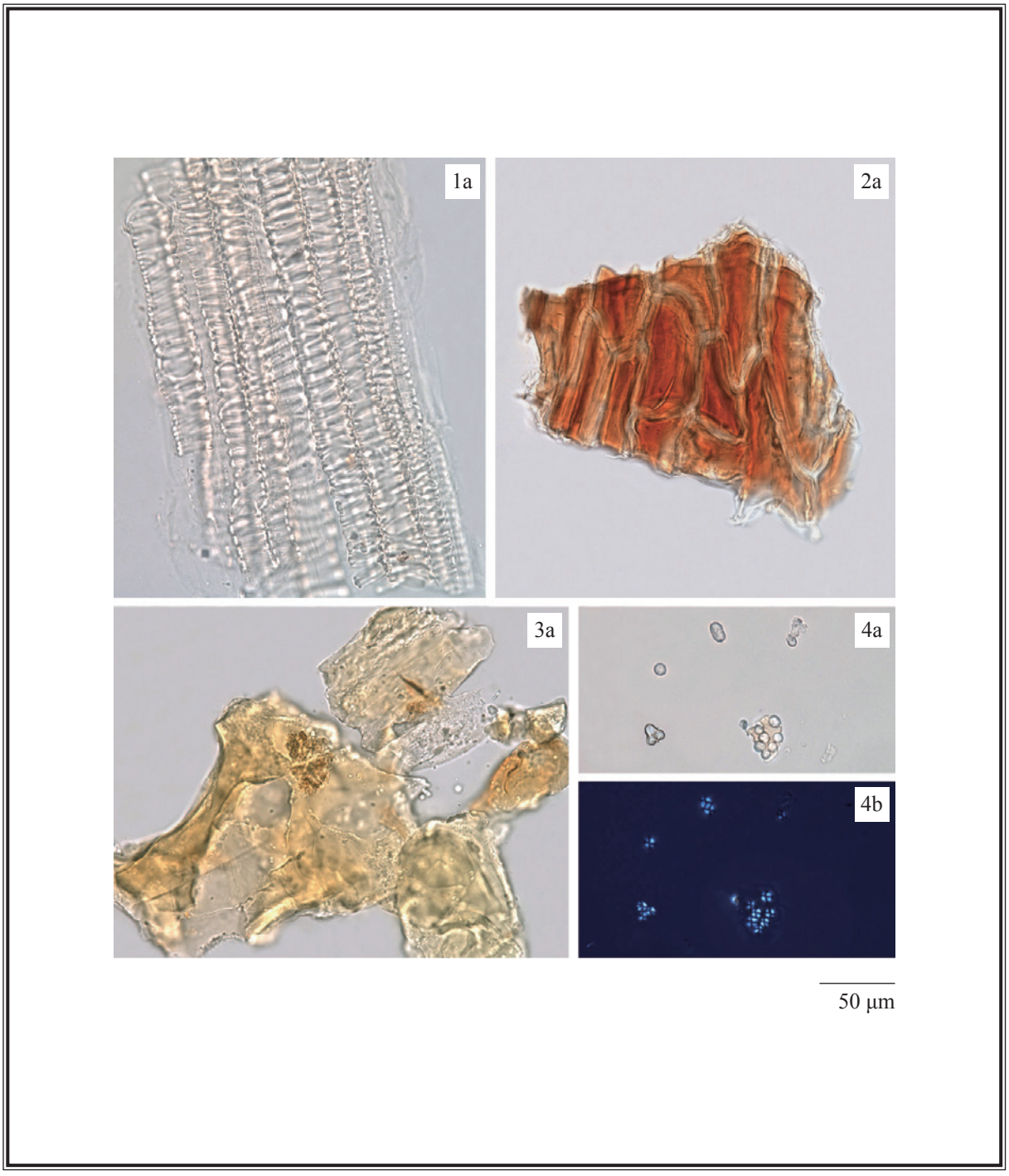

Figure 3 Microscopic features of powder of Rhodiolae Crenulatae Radix et Rhizoma

1. Vessels 2. Cork cells
3. Parenchymatous cell with yellowish-brown secretions 4. Starch granules

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

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

Standard solution

p-Hydroxyphenethyl glucopyranoside standard solution

Weigh 1.0 mg of *p*-hydroxyphenethyl glucopyranoside CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane and methanol (5:1, v/v).

Spray reagent

Add slowly 5 mL of sulphuric acid to 45 mL of ethanol and dissolve 0.5 g of vanillin.

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 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

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 *p*-hydroxyphenethyl glucopyranoside standard solution and the test solution (2 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 7 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 and heat at about 110ºC until the spots or bands become visible (about 10-15 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

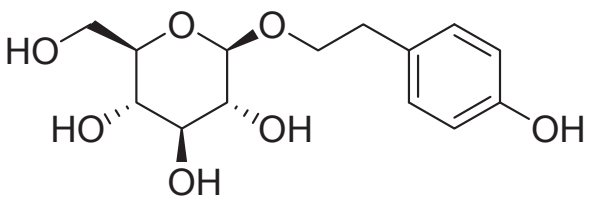

Figure 4 Chemical structure of *p*-hydroxyphenethyl glucopyranoside

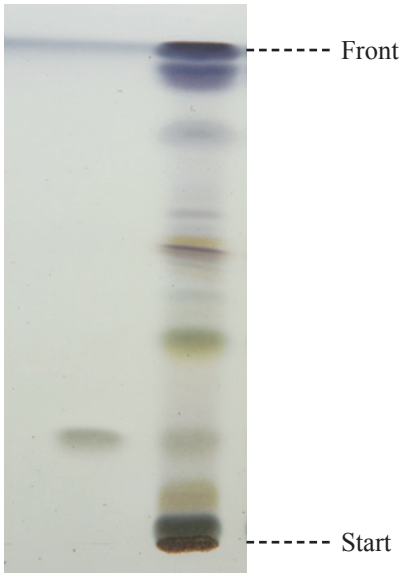

Figure 5 A reference HPTLC chromatogram of Rhodiolae Crenulatae Radix et Rhizoma extract observed under visible light after staining

1. *p*-Hydroxyphenethyl glucopyranoside 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 p-hydroxyphenethyl glucopyranoside (Fig. 5).

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

Standard solution

p-Hydroxyphenethyl glucopyranoside standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of *p*-hydroxyphenethyl glucopyranoside CRS and dissolve in 10 mL of water.

Test solution

Weigh 1.0 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 (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at

20ºC during the separation. 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)	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 *p*-hydroxyphenethyl glucopyranoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of *p*-hydroxyphenethyl glucopyranoside should not be more than 5.0%; the RSD of the retention time of *p*-hydroxyphenethyl glucopyranoside peak should not be more than 2.0%; the column efficiency determined from *p*-hydroxyphenethyl glucopyranoside peak should not be less than 15000 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 *p*-hydroxyphenethyl glucopyranoside Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of *p*-hydroxyphenethyl glucopyranoside peak in the chromatogram of *p*-hydroxyphenethyl glucopyranoside Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify *p*-hydroxyphenethyl glucopyranoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of *p*-hydroxyphenethyl glucopyranoside Std-FP. The retention times of *p*-hydroxyphenethyl glucopyranoside 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 Rhodiolae Crenulatae Radix et Rhizoma extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.68	± 0.03
2 (marker, *p*-hydroxyphenethyl glucopyranoside)	1.00	-
3	1.40	± 0.04
4	2.52	± 0.05

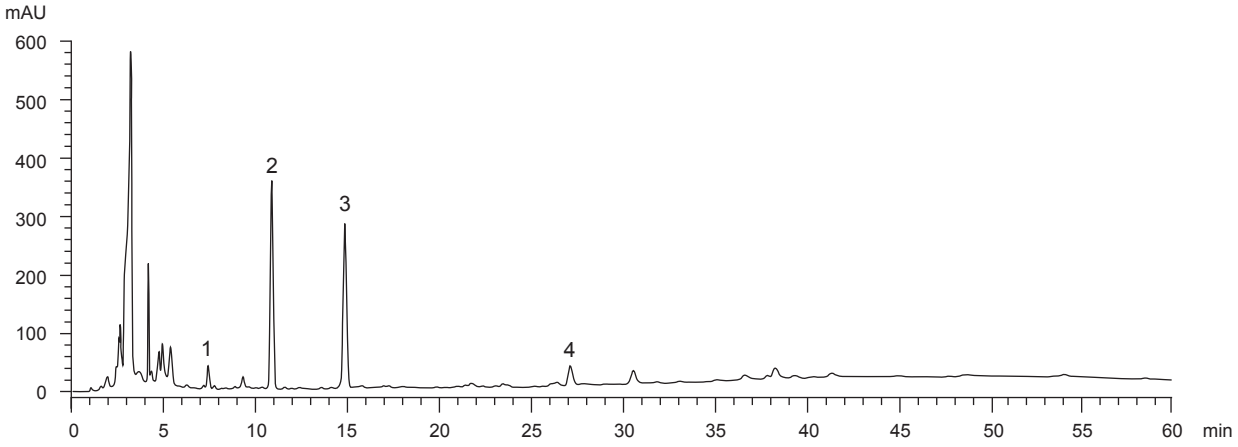

Figure 6 A reference fingerprint chromatogram of Rhodiolae Crenulatae Radix et Rhizoma extract

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 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 1.0%.

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 14.0%.
Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

p-Hydroxyphenethyl glucopyranoside standard stock solution, Std-Stock (1000 mg/L)
Weigh accurately 5.0 mg of *p*-hydroxyphenethyl glucopyranoside CRS and dissolve in 5 mL of water.
p-Hydroxyphenethyl glucopyranoside standard solution for assay, Std-AS
Measure accurately the volume of the *p*-hydroxyphenethyl glucopyranoside Std-Stock, dilute with water to produce a series of solutions of 10, 150, 300, 400, 500 mg/L for *p*-hydroxyphenethyl glucopyranoside.

Test solution

Weigh accurately 1.0 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 (220 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 20ºC during the separation. 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)	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 *p*-hydroxyphenethyl glucopyranoside Std-AS (300 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of *p*-hydroxyphenethyl glucopyranoside should not be more than 5.0%; the RSD of the retention time of *p*-hydroxyphenethyl glucopyranoside peak should not be more than 2.0%; the column efficiency determined from *p*-hydroxyphenethyl glucopyranoside peak should not be less than 15000 theoretical plates.

The *R* value between *p*-hydroxyphenethyl glucopyranoside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of *p*-hydroxyphenethyl glucopyranoside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of *p*-hydroxyphenethyl glucopyranoside against the corresponding concentrations of *p*-hydroxyphenethyl glucopyranoside Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify *p*-hydroxyphenethyl glucopyranoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of *p*-hydroxyphenethyl glucopyranoside Std-AS. The retention times of *p*-hydroxyphenethyl glucopyranoside 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 *p*-hydroxyphenethyl glucopyranoside in the test solution, and calculate the percentage content of *p*-hydroxyphenethyl glucopyranoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.50% of *p*-hydroxyphenethyl glucopyranoside ($C_{14}H_{20}O_7$), calculated with reference to the dried substance.