Scrophulariae Radix

Figure 1 A photograph of Scrophulariae Radix

1. NAMES

Official Name: Scrophulariae Radix

Chinese Name: 玄參

Chinese Phonetic Name: Xuanshen

2. SOURCE

Scrophulariae Radix is the dried root of *Scrophularia ningpoensis* Hemsl. (Scrophulariaceae). The root is collected in winter, removed rhizome, rootlets, root buds, soil and sand, slowly baked or dried under the sun until semi-dried, then piled for 3-6 days; repeat several times to dry, then obtain Scrophulariae Radix.

3. DESCRIPTION

Subcylindrical, slightly thick at the middle part or thick at the upper part and slender at the lower part; sometimes slightly curved, 6-28 cm long, 6-40 mm in diameter. Externally greyish-yellow or greyish-brown, with distinct, irregular longitudinal grooves and latitudinal lenticels; scars of rootlet occasionally observed. Texture tough, pliable, transverse section black and slightly lustrous. Odour caramel-like; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Metaderm slightly suberized. Cortex relatively broad; stone cells numerous, polygonal, subrounded or subsquare in shape; cell walls thick, mostly singly scattered or 2-5 in groups. Phloem slightly narrow. Cambium in a ring. Xylem ray broad, vessels arranged intermittently and radially, those in the inter part of xylem surrounded with xylem fibres. Metaxylem observed in the centre. Parenchymatous cells contain nucleus-like contents (Fig. 2).

Powder

Colour greyish-yellow to greyish-brown. Stone cells numerous, mostly singly scattered or 2-5 in groups; rectangular, subsquare or subrounded, triangular or fusiform in shape, 23-132 μm in diameter or 47-250 μm long, cell walls 4-22 μm thick; pits small, pit canals mostly ramified,

lumina relatively large. Parenchymatous cells numerous, mostly containing dark nucleus-like contents. Fibres slender, slightly lignified, with small pits. Reticulate and bordered-pitted vessels, 4-125 µm. Metaderm cells brownish-yellow, subrectangular on the surface view; walls slightly thickened and suberized (Fig. 3).

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

Standard solutions

Harpagide standard solution

Weigh 0.5 mg of harpagide CRS (Fig. 4) and dissolve in 1 mL of methanol.

Harpagoside standard solution

Weigh 0.5 mg of harpagoside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, methanol and formic acid (4:1:0.1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

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 (50%). Sonicate (90 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately harpagide standard solution (1 µL), harpagoside standard solution (0.5 µL) and the test solution (3 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. Spray the plate evenly with the spray reagent and heat it at about 105°C until the spots or bands become visible (about 1 min). 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 harpagide and harpagoside.

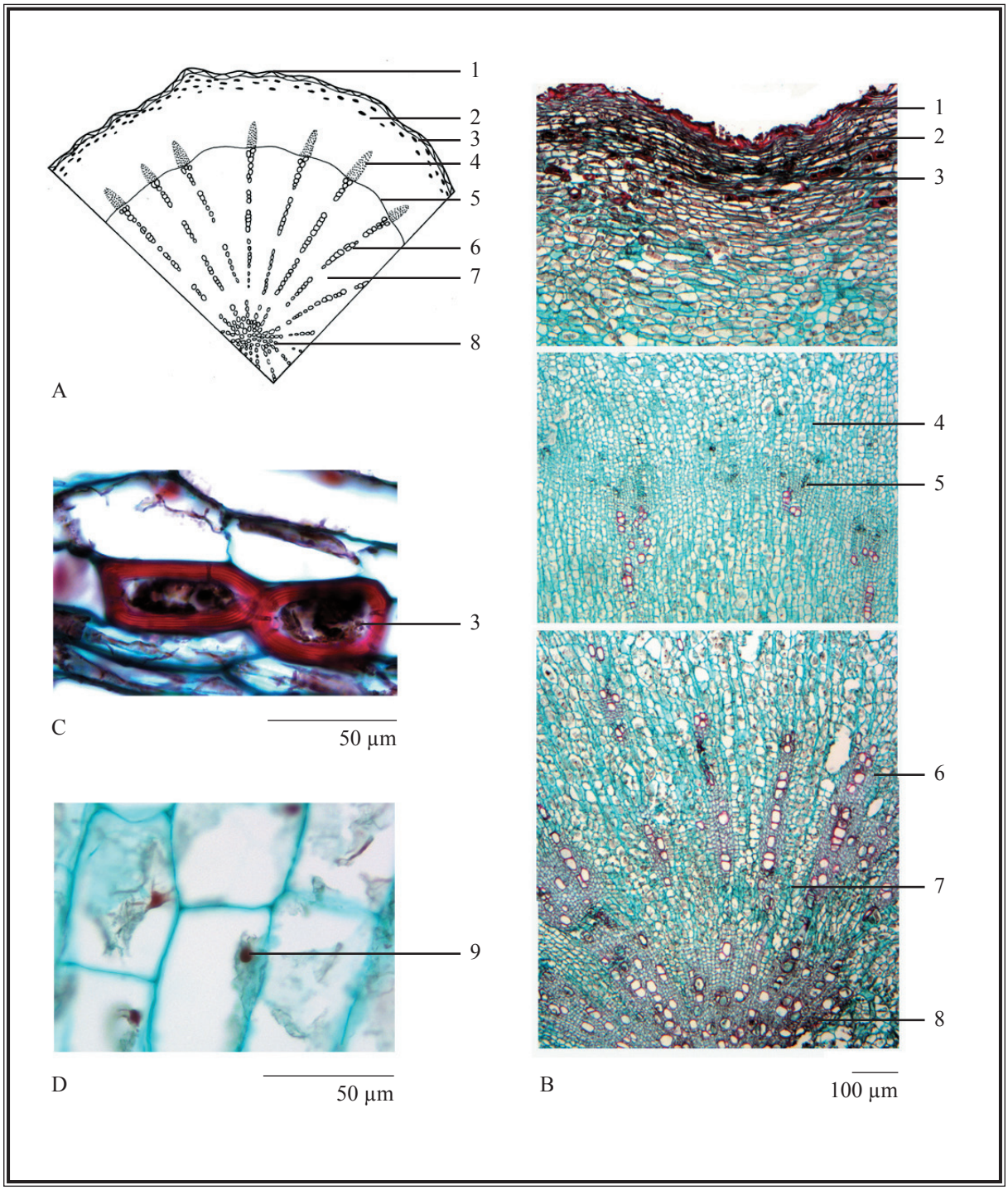


Figure 2 Microscopic features of transverse section of Scrophulariae Radix

A. Sketch B. Section illustration C. Stone cells D. Parenchymatous cells

1. Metaderm 2. Cortex 3. Stone cells 4. Phloem 5. Cambium 6. Xylem 7. Ray
8. Metaxylem 9. Nucleus-like content

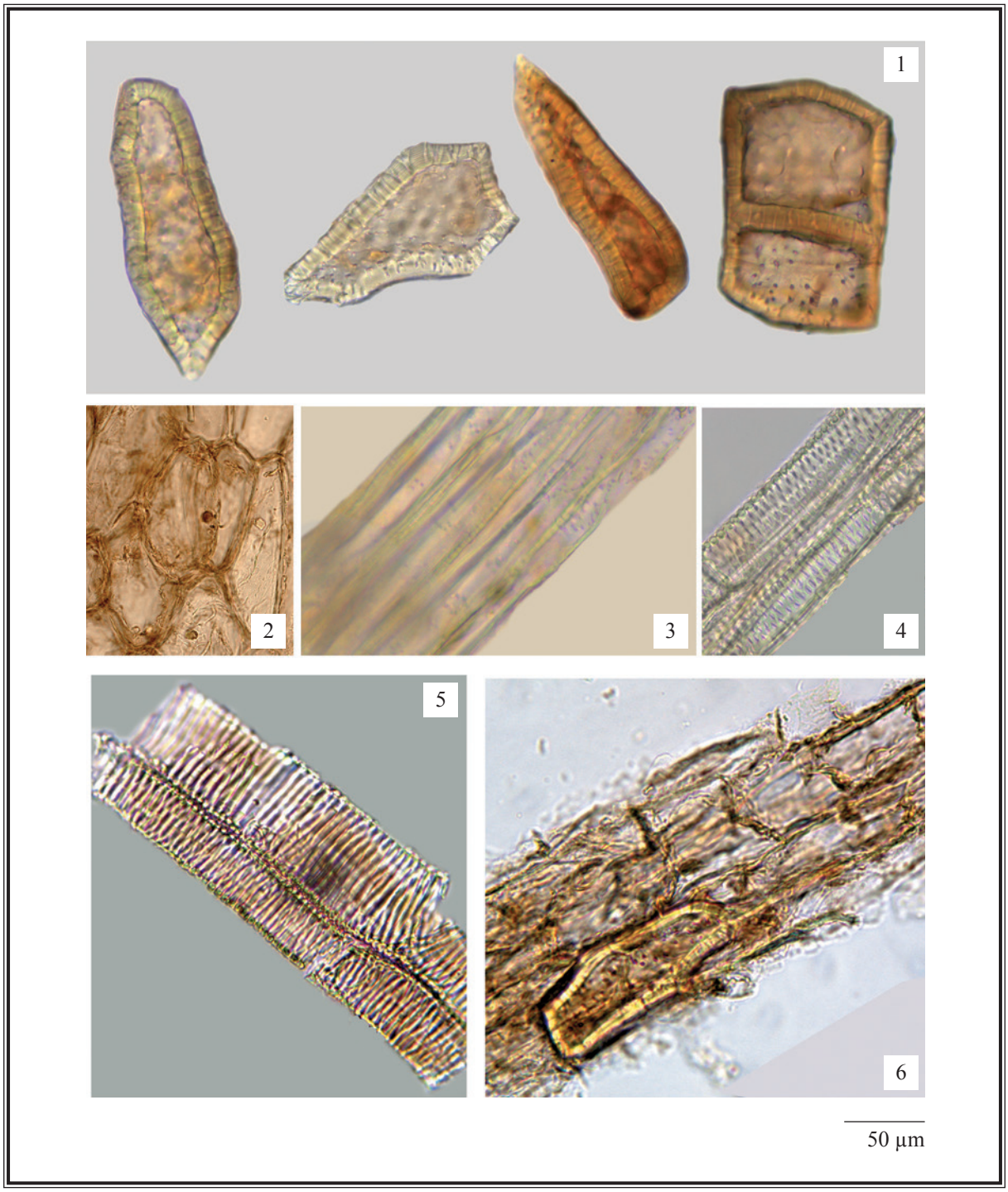


Figure 3 Microscopic features of powder of Scrophulariae Radix (under the light microscope)

1. Stone cells 2. Parenchymatous cells 3. Fibres 4. Bordered-pitted vessels
5. Reticulate-scalariform vessels 6. Metaderm cells

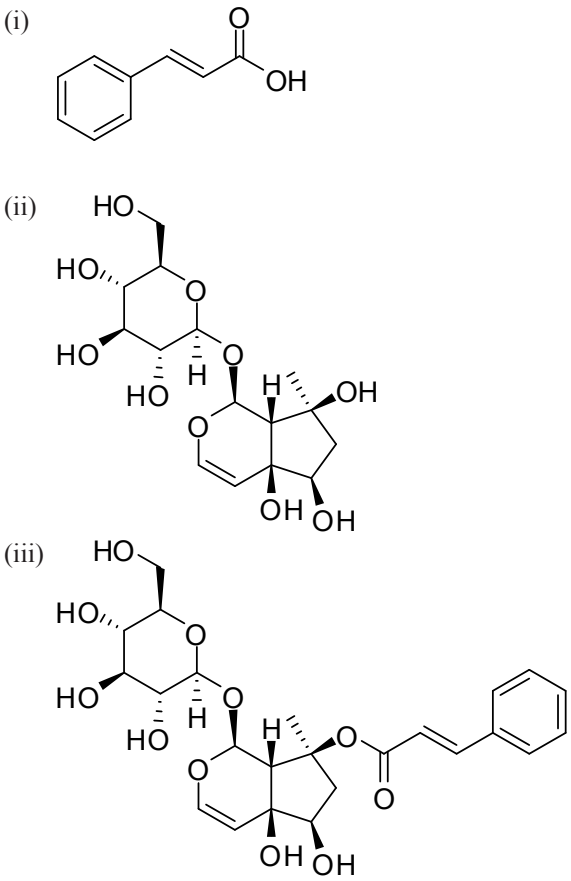


Figure 4 Chemical structures of (i) cinnamic acid (ii) harpagide and (iii) harpagoside

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

Standard solutions

Cinnamic acid standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.5 mg of cinnamic acid CRS (Fig. 4) and dissolve in 25 mL of ethanol (50%).

Harpagoside standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 2.5 mg of harpagoside CRS and dissolve in 25 mL of ethanol (50%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Sonicate (90 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 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)	Methanol (%, v/v)	0.05% Trifluoroacetic acid (%, v/v)	Elution
0 – 10	40	60	isocratic
10 – 30	40 → 70	60 → 30	linear gradient
30 – 40	70	30	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of cinnamic acid Std-FP and harpagoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamic acid and harpagoside should not be more than 5.0%; the RSD of the retention times of cinnamic acid and harpagoside peaks should not be more than 2.0%; the column efficiencies determined from cinnamic acid and harpagoside peaks should not be less than 80000 and 110000 theoretical plates respectively.

The *R* value between peak 2 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 cinnamic acid Std-FP, harpagoside Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of cinnamic acid and harpagoside peaks in the chromatograms of cinnamic acid Std-FP, harpagoside Std-FP and the retention times of the three characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify cinnamic acid and harpagoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of cinnamic acid Std-FP and harpagoside Std-FP. The retention times of cinnamic acid and harpagoside 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 three characteristic peaks of Scrophulariae Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Scrophulariae Radix extract

Peak No.	RRT	Acceptable Range
1	0.76	± 0.03
2 (marker, cinnamic acid)	1.00	-
3 (harpagoside)	1.16	± 0.03

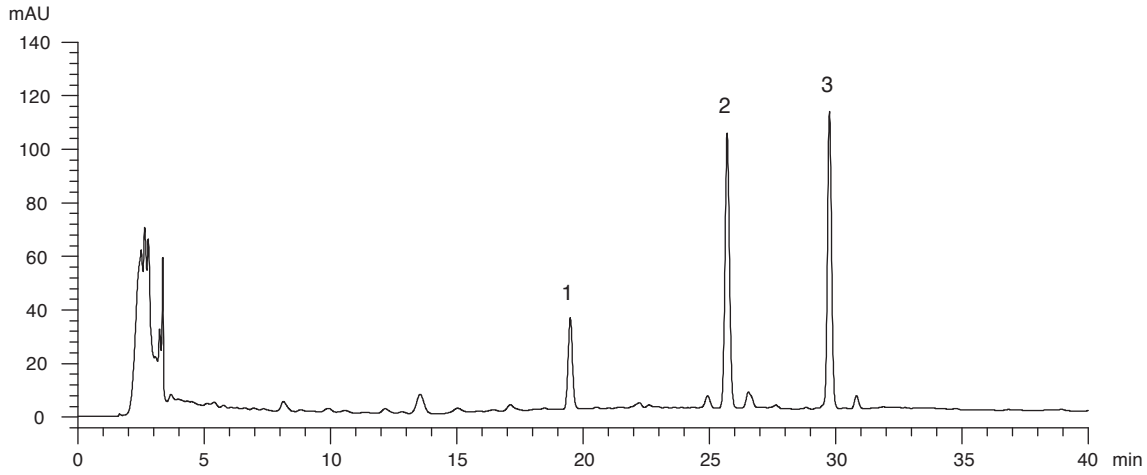


Figure 5 A reference fingerprint chromatogram of Scrophulariae Radix extract

For positive identification, the sample must give the above three 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 – Aflatoxins *(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 5.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 16.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 60.0%.
Ethanol-soluble extractives (cold extraction method): not less than 57.0 %.

7. ASSAY

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

Standard solution

Mixed cinnamic acid, harpagide and harpagoside standard stock solution, Std-Stock (40 mg/L for cinnamic acid, 400 mg/L for harpagide and 160 mg/L for harpagoside)

Weigh accurately 0.4 mg of cinnamic acid CRS, 4.0 mg of harpagide CRS, 1.6 mg of harpagoside CRS, and dissolve in 10 mL of ethanol (30%).

Mixed cinnamic acid, harpagide and harpagoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed cinnamic acid, harpagide and harpagoside Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 2, 6, 12, 16, 20 mg/L for cinnamic acid, 20, 60, 120, 160, 200 mg/L for harpagide and 8, 24, 48, 64, 80 mg/L for harpagoside.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (30%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (30%). Combine the solutions and make up to the mark with ethanol (30%). Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm for harpagide; 280 nm for cinnamic acid and harpagoside) 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)	0.01% Trifluoroacetic acid (%, v/v)	Elution
0 – 10	3 → 10	97 → 90	linear gradient
10 – 20	10 → 33	90 → 67	linear gradient
20 – 25	33 → 50	67 → 50	linear gradient
25 – 30	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 µL of the mixed cinnamic acid, harpagide and harpagoside Std-AS (16 mg/L for cinnamic acid, 160 mg/L for harpagide and 64 mg/L for harpagoside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of cinnamic acid, harpagide and harpagoside should not be more than 5.0%; the RSD of the retention times of cinnamic acid, harpagide and harpagoside peaks should not be more than 2.0%; the column efficiencies determined from cinnamic acid, harpagide and harpagoside peaks should not be less than 220000, 30000 and 250000 theoretical plates respectively.

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

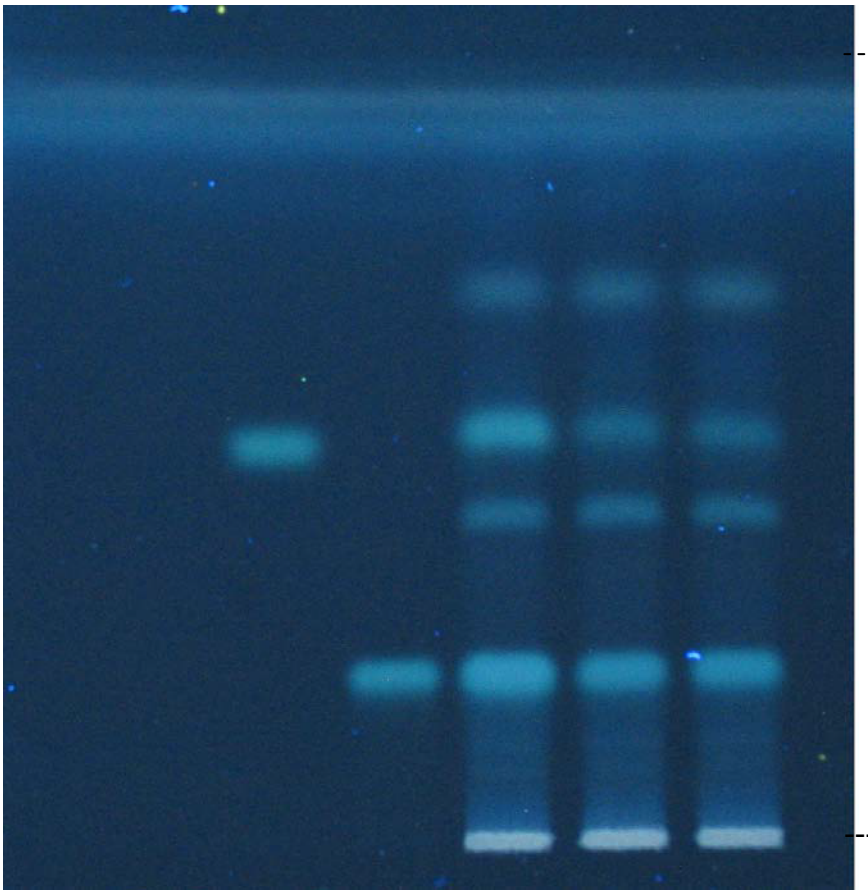
Procedure

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

Limits

The sample contains not less than 0.030% of cinnamic acid ($C_9H_8O_2$) and not less than 0.45% of the total content of harpagide ($C_{15}H_{24}O_{10}$) and harpagoside ($C_{24}H_{30}O_{11}$), calculated with reference to the dried substance.

Scrophulariae Radix　(玄参)



Lane	Sample	Results
1	Blank (50% ethanol)	Negative
2	Standard (Harpagoside)	Harpagoside positive
3	Standard (Harpagide)	Harpagide positive
4	Spiked sample (Sample plus harpagoside and harpagide)	Harpagoside and harpagide positive
5	Sample (Scrophulariae Radix)	Harpagoside and harpagide positive
6	Sample duplicate (Scrophulariae Radix)	Harpagoside and harpagide positive

Figure 1　TLC results of Scrophulariae Radix extract observed under UV light (366 nm) after staining