Wikstroemiae Radix

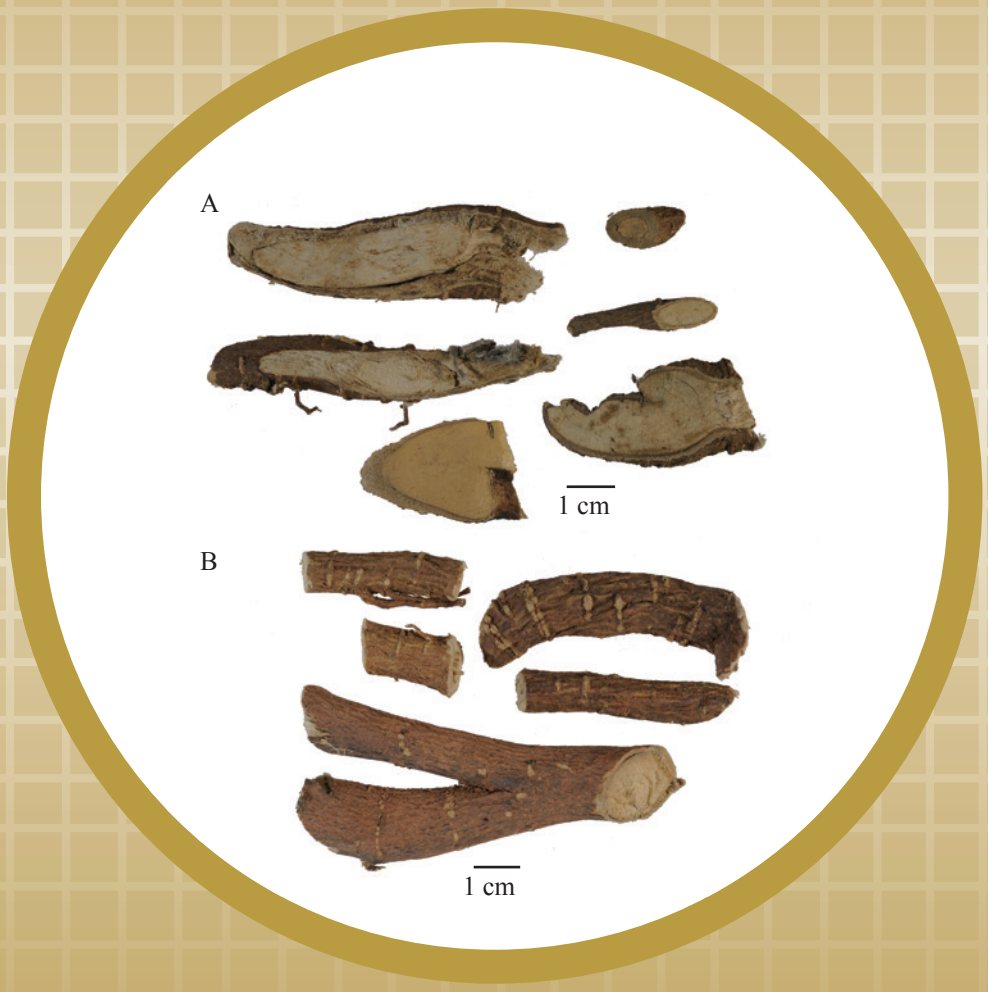

Figure 1 A photograph of Wikstroemiae Radix

A. Oblique slices of Wikstroemiae Radix

B. Short pieces of Wikstroemiae Radix

1. NAMES

Official Name: Wikstroemiae Radix

Chinese Name: 了哥王

Chinese Phonetic Name: Liaogewang

2. SOURCE

Wikstroemiae Radix is the dried root of *Wikstroemia indica* (L.) C. A. Mey. (Thymelaeaceae). The root is collected in autumn to early spring, washed clean, foreign matter removed, cut into short pieces or oblique slices while fresh, then dried under the sun to obtain Wikstroemiae Radix.

3. DESCRIPTION

Short pieces or oblique slices. Short pieces cylindrical, often branched, 1-13 cm long, 4-50 mm in diameter; oblique slices 1-12.5 cm long, 0.5-7.5 cm wide, 0.2-0.4 cm thick. Externally yellowish-brown to pale brown, with longitudinal wrinkles, transverse elongated lenticels and small protruding rootlet scars. Texture hard and flexible, fracture greyish-yellow, the root bark easily stripped from the wood; Odour slight; taste slightly bitter, pungent on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of rectangular suberized cells. Cortex scattered with abundant fibres. Phloem broad, scattered with abundant fibres. Phloem ray consists of 1-3 rows of cells, radially elongated and arranged radially. Cambium consists of 1 layer of cells, arranged in a ring. Xylem vessels singly scattered or several in groups. Xylem ray consists of 1-3 rows of cells, radially elongated and arranged radially (Fig. 2).

Powder

Colour pale yellowish-brown. Fibres abundant, mostly scattered singly, long fusiform, easily broken, 8-45 μm in diameter; bluish-white under the polarized microscope. Starch granules few, mostly simple starch granules, subrounded to elliptic, 4-35 μm in diameter, hilum dotted and locate on a side; black and cruciate-shaped under the polarized microscope. Vessels mainly reticulate, easily broken. Cork cells polygonal in surface view, sometimes contain yellowish-brown or brownish-red resinous contents. Crystals of calcium oxalate few, singly scattered, varying in shape; yellowish-white or polychromatic under the polarized microscope (Fig. 3).

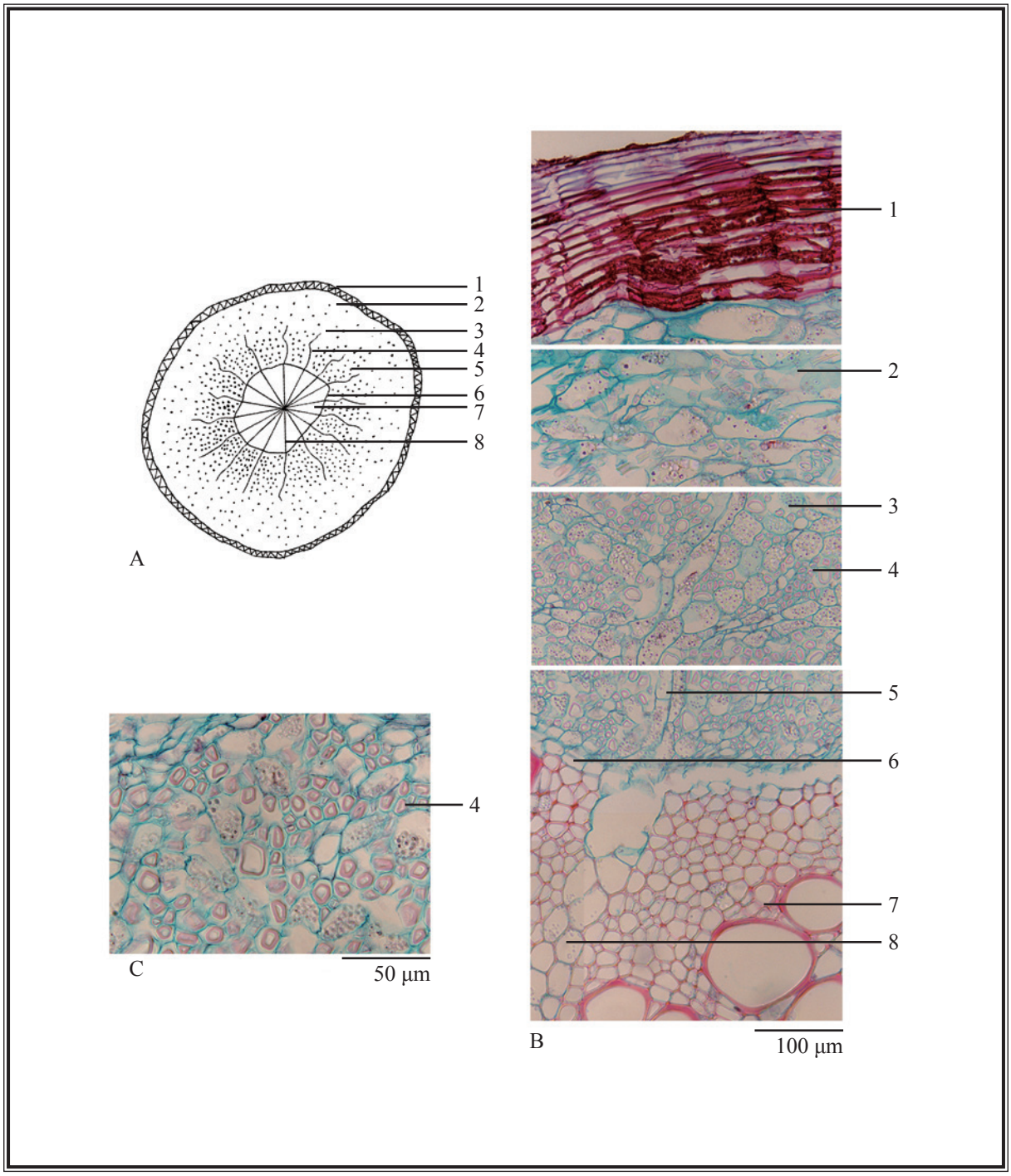

Figure 2 Microscopic features of transverse section of Wikstroemiae Radix

A. Sketch B. Section illustration C. Fibres

1. Cork 2. Cortex 3. Phloem 4. Fibres 5. Phloem ray 6. Cambium 7. Xylem 8. Xylem ray

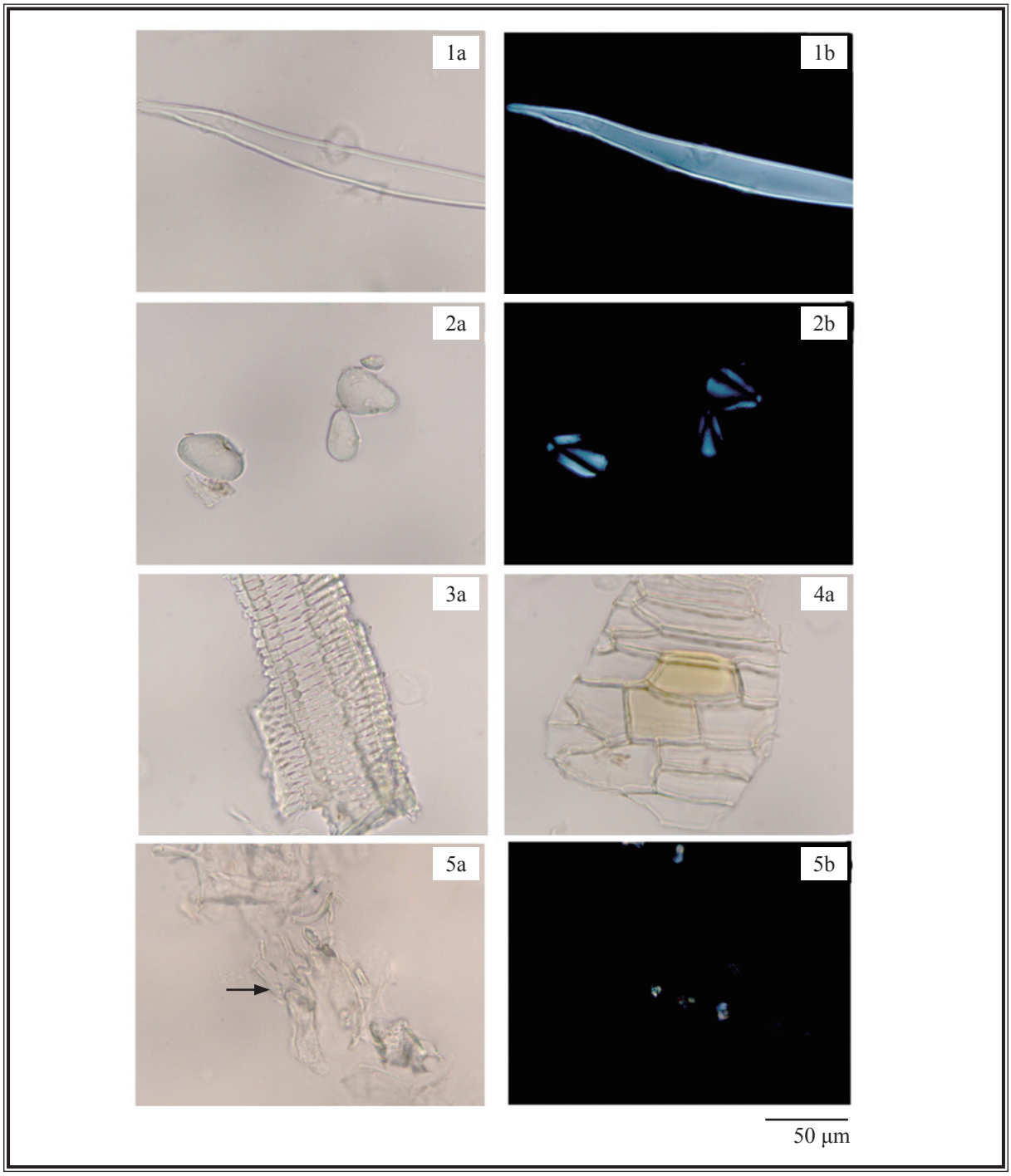

Figure 3 Microscopic features of powder of Wikstroemiae Radix

1. Fibre 2. Starch granules 3. Reticulate vessls 4. Cork cells 5. Crystals of calcium oxalate (→)

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

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

Standard solution

Daphnoretin standard solution

Weigh 2.0 mg of daphnoretin CRS (Fig. 4) and dissolve in 10 mL of methanol.

Developing solvent system

Prepare a mixture of methanol and water (4:1, 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 (150 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a TLC polyamide plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately daphnoretin standard solution (1 μL) and the test solution (2 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC 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. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

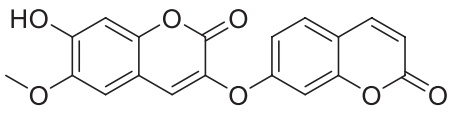

Figure 4 Chemical structure of daphnoretin

Figure 5 A reference TLC chromatogram of Wikstroemiae Radix extract observed under UV light (366 nm)

1. Daphnoretin 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 daphnoretin (Fig. 5).

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

Standard solution

Daphnoretin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of daphnoretin CRS and dissolve in 10 mL of acetonitrile.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (70%). Sonicate (150 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (320 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)	1% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	80 → 77	20 → 23	linear gradient
10 – 20	77 → 71	23 → 29	linear gradient
20 – 30	71	29	isocratic
30 – 45	71 → 60	29 → 40	linear gradient
45 – 50	60 → 58	40 → 42	linear gradient
50 – 60	58	42	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of daphnoretin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of daphnoretin should not be more than 5.0%; the RSD of the retention time of daphnoretin peak should not be more than 2.0%; the column efficiency determined from daphnoretin peak should not be less than 20000 theoretical plates.

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. 6).

Procedure

Separately inject daphnoretin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of daphnoretin peak in the chromatogram of daphnoretin Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify daphnoretin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of daphnoretin Std-FP. The retention times of daphnoretin 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 six characteristic peaks of Wikstroemiae Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Wikstroemiae Radix extract

Peak No.	RRT	Acceptable Range
1	0.44	± 0.03
2	0.67	± 0.03
3 (marker, daphnoretin)	1.00	-
4	1.39	± 0.03
5	1.61	± 0.04
6	1.75	± 0.03

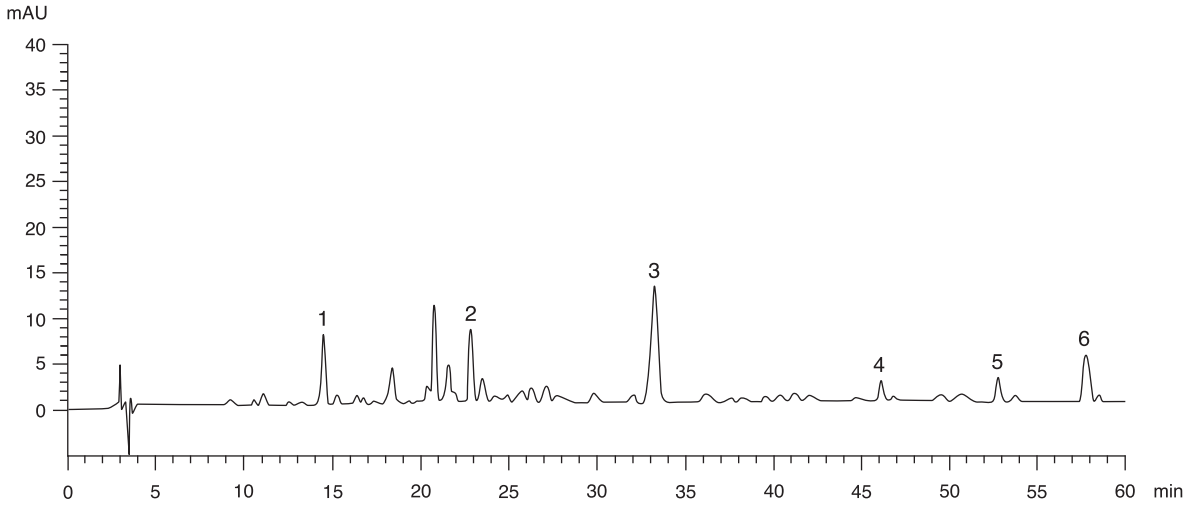

Figure 6 A reference fingerprint chromatogram of Wikstroemiae Radix extract

For positive identification, the sample must give the above six 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)*: The CMM shall meet the requirements for arsenic, lead and mercury as specified in Appendix V. For cadmium, Wikstroemiae Radix should meet the specified limit of not more than 13.5 mg/kg, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limit for cadmium specified in Appendix V shall be applied.

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

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: 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 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Daphnoretin standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of daphnoretin CRS and dissolve in 10 mL of acetonitrile.
Daphnoretin standard solution for assay, Std-AS
Measure accurately the volume of the daphnoretin Std-Stock, dilute with acetonitrile to produce a series of solutions of 1, 2.5, 5, 10, 25 mg/L for daphnoretin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (70%). 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 one more time. Wash the residue with methanol (70%). Combine the solutions and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 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 3) –

Table 3 Chromatographic system conditions

Time (min)	1% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	70	30	isocratic
10 – 20	70 → 65	30 → 35	linear gradient
20 – 40	65 → 20	35 → 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of daphnoretin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of daphnoretin should not be more than 5.0%; the RSD of the retention time of daphnoretin peak should not be more than 2.0%; the column efficiency determined from daphnoretin peak should not be less than 8000 theoretical plates.

The *R* value between daphnoretin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of daphnoretin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of daphnoretin against the corresponding concentrations of daphnoretin 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 daphnoretin peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of daphnoretin Std-AS. The retention times of daphnoretin 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 daphnoretin in the test solution, and calculate the percentage content of daphnoretin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.095% of daphnoretin ($C_{19}H_{12}O_7$), calculated with reference to the dried substance.

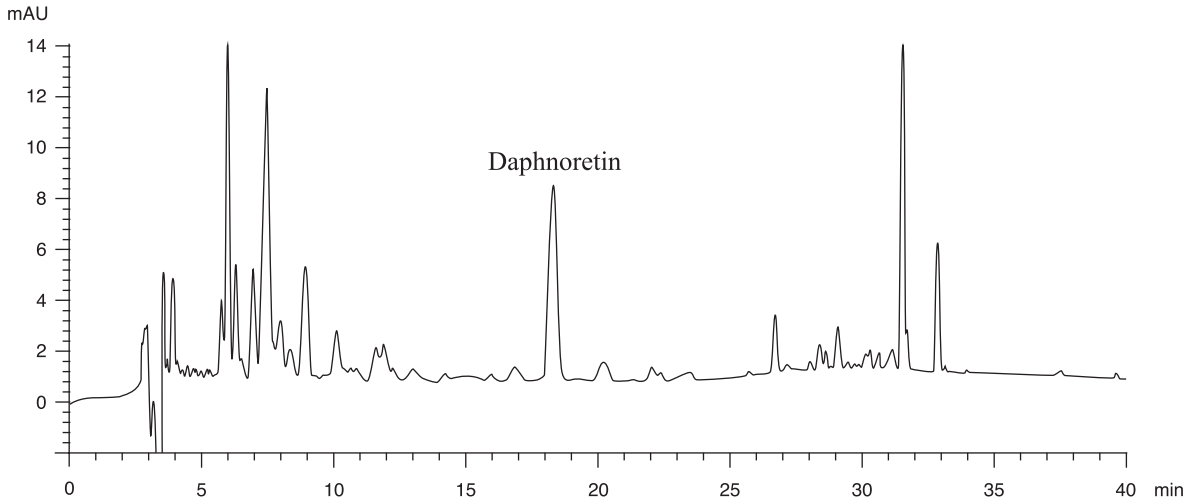

Figure 7 A reference assay chromatogram of Wikstroemiae Radix extract

8. CAUTION

This CMM should be used after proper processing (such as decoction).