Semiaquilegiae Radix

Figure 1 A photograph of Semiaquilegiae Radix

A. Semiaquilegiae Radix (rootlet scar →)

B. Magnified image of cut surface of root tuber

1. NAMES

Official Name: Semiaquilegiae Radix

Chinese Name: 天葵子

Chinese Phonetic Name: Tiankuizi

2. SOURCE

Semiaquilegiae Radix is the dried root tuber of *Semiaquilegia adoxoides* (DC.) Makino (Ranunculaceae). The root tuber is collected in early summer, washed clean, rootlets removed, then dried under the sun to obtain Semiaquilegiae Radix.

3. DESCRIPTION

Irregularly short-cylindrical, fusiform or lump-shaped, slightly curved, 1.0-4.7 cm long, 3-15 mm in diameter. Externally dark brown, blackish-brown to greyish-black, with irregular longitudinal wrinkles and rootlet scars. Apex usually with remains of stems or leaves, covered with several layers of yellowish-brown sheath-like scales. Texture relatively soft, easily broken, transversely cut surface whitish or pale yellowish-white in the bark and yellowish-white to yellowish-brown and slightly radially striated in the wood. Odour slight; taste sweet, slightly bitter and pungent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Cortex broad. Phloem arranged radially in a narrow-conical shape, the tip reaching nearly to the outer edge of the cortex. Cambium in a ring. Xylem rays consist of over 20 rows of cells at the interphase with the cambium; vessels arranged radially, sparsely scattered in the centre of root (Fig. 2).

Powder

Colour greyish-brown to blackish-brown. Starch granules rare, mainly simple, broadly ovoid, long-spheroidal or irregularly spheroidal, 4-32 μm in diameter; striations mostly indistinct; black and cruciate-shaped under the polarized microscope. Xylem ray cells subsquare, subrectangular or subpolygonal, with slightly thickened walls. Vessels mostly reticulate, 8-41 μm in diameter. Cork cells subsquare, subrectangular or irregular in shape, with thickened walls, usually containing yellowish-brown to reddish-brown contents (Fig. 3).

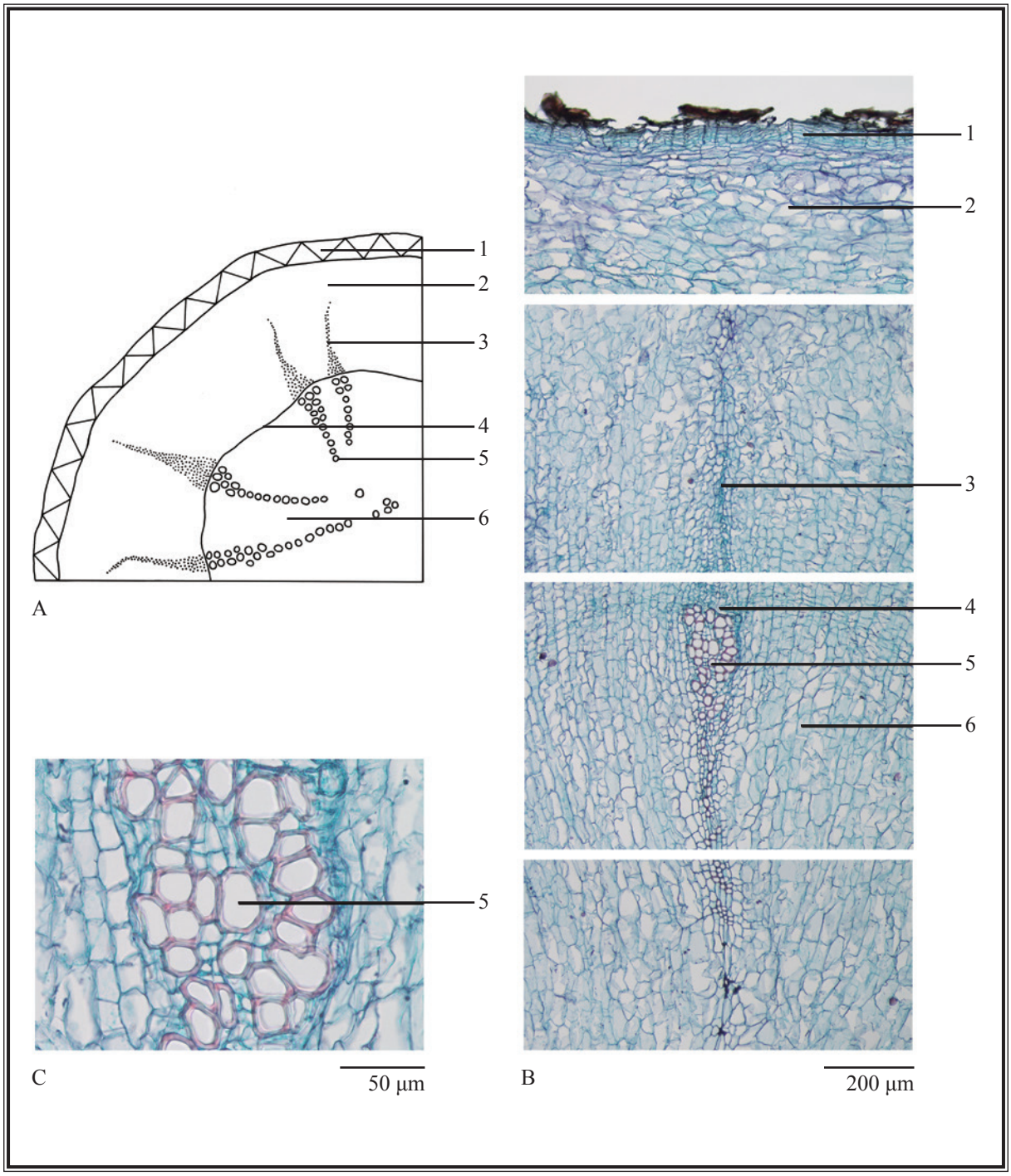

Figure 2 Microscopic features of transverse section of Semiaquilegiae Radix

A. Sketch B. Section illustration C. Vessels

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

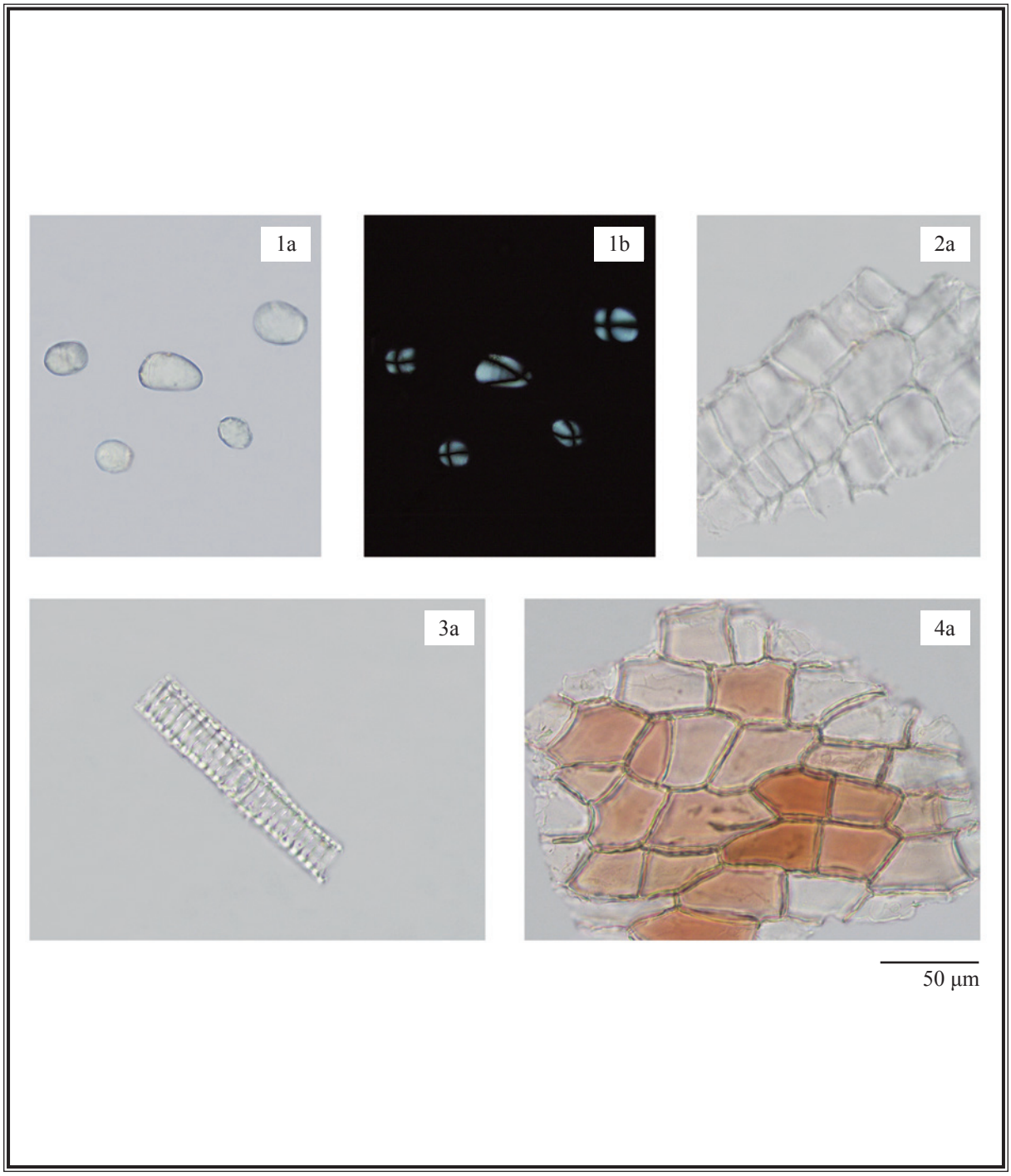

Figure 3 Microscopic features of powder of Semiaquilegiae Radix

1. Starch granules 2. Xylem ray cells 3. Reticulate vessel 4. Cork cells

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

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

Standard solutions

Griffonilide standard solution

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

Lithospermoside (griffonin) standard solution

Weigh 0.25 mg of lithospermoside CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and water (7:3:0.5, 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 (400 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 5 mL of methanol. Filter through a 0.45-μm nylon filter.

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 griffonilide standard solution (1 μL), lithospermoside standard solution (5 μL) and the test solution (5 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. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

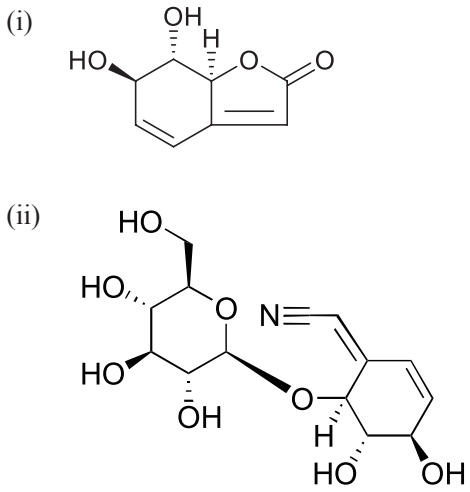

Figure 4 Chemical structures of (i) griffonilide and (ii) lithospermoside (griffonin)

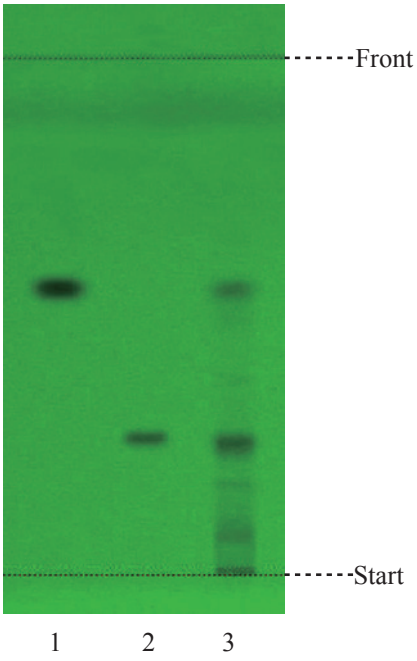

Figure 5 A reference HPTLC chromatogram of Semiaquilegiae Radix extract observed under UV light (254 nm)

1. Griffonilide standard solution 2. Lithospermoside 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 griffonilide and lithospermoside (Fig. 5).

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

Standard solutions

Griffonilide standard solution for fingerprinting, Std-FP (3 mg/L)

Weigh 0.15 mg of griffonilide CRS and dissolve in 50 mL of ethanol (50%).

Lithospermoside (griffonin) standard solution for fingerprinting, Std-FP (6 mg/L)

Weigh 0.3 mg of lithospermoside CRS and dissolve in 50 mL of ethanol (50%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 15 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (258 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	100	0	isocratic
5 – 30	100 → 50	0 → 50	linear gradient
30 – 40	50 → 10	50 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of griffonilide Std-FP and lithospermoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of griffonilide and lithospermoside should not be more than 5.0%; the RSD of the retention times of griffonilide and lithospermoside peaks should not be more than 2.0%; the column efficiencies determined from griffonilide and lithospermoside peaks should not be less than 150000 and 100000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and 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 griffonilide Std-FP, lithospermoside Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention times of griffonilide and lithospermoside peaks in the chromatograms of griffonilide Std-FP, lithospermoside Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify griffonilide and lithospermoside peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of griffonilide Std-FP and lithospermoside Std-FP. The retention times of griffonilide and lithospermoside 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 six characteristic peaks of Semiaquilegiae Radix extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, lithospermoside)	1.00	-
2 (griffonilide)	1.04	± 0.03
3	1.12	± 0.03
4	1.31	± 0.03
5	1.41	± 0.03
6	1.43	± 0.03

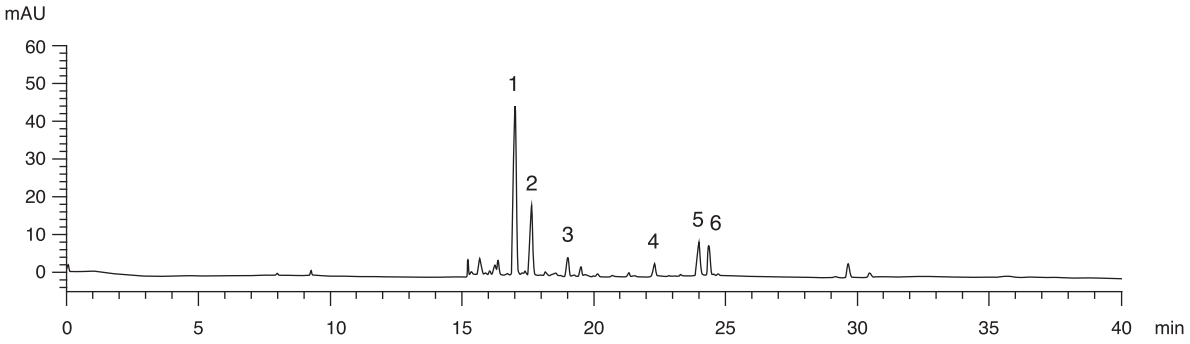

Figure 6 A reference fingerprint chromatogram of Semiaquilegiae Radix extract

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)*: 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 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 6.0%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Contentt *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 48.0%.
Ethanol-soluble extractives (cold extraction method): not less than 27.0%.

7. ASSAY

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

Standard solution

Mixed griffonilide and lithospermoside (griffonin) standard stock solution, Std-Stock (200 mg/L each)
Weigh accurately 5.0 mg of griffonilide CRS and 5.0 mg of lithospermoside CRS, and dissolve in 25 mL of ethanol (50%).
Mixed griffonilide and lithospermoside standard solution for assay, Std-AS
Measure accurately the volume of the mixed griffonilide and lithospermoside Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.4, 0.8, 1.5, 3, 6 mg/L for griffonilide and 1.5, 3, 6, 12, 24 mg/L for lithospermoside.

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 ethanol (50%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 15 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (50%). Combine the solutions and make up to the mark with ethanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (258 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.6 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	100	0	isocratic
5 – 30	100 → 50	0 → 50	linear gradient
30 – 40	50 → 10	50 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed griffonilide and lithospermoside Std-AS (1.5 mg/L for griffonilide and 6 mg/L for lithospermoside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of griffonilide and lithospermoside should not be more than 5.0%; the RSD of the retention times of griffonilide and lithospermoside peaks should not be more than 2.0%; the column efficiencies determined from griffonilide and lithospermoside peaks should not be less than 150000 and 100000 theoretical plates respectively.

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

Calibration curves

Inject a series of the mixed griffonilide and lithospermoside Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of griffonilide and lithospermoside against the corresponding concentrations of the mixed griffonilide and lithospermoside 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 griffonilide and lithospermoside peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed griffonilide and lithospermoside Std-AS. The retention times of griffonilide and lithospermoside 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 griffonilide and lithospermoside in the test solution, and calculate the percentage contents of griffonilide and lithospermoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.030% of griffonilide ($C_8H_8O_4$) and not less than 0.10% of lithospermoside ($C_{14}H_{19}NO_8$), calculated with reference to the dried substance.

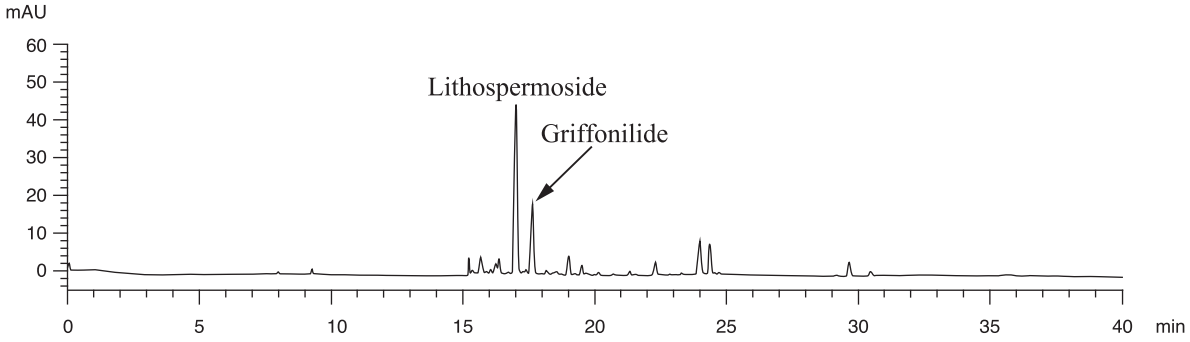

Figure 7 A reference assay chromatogram of Semiaquilegiae Radix extract