

Radix Puerariae Thomsonii



Figure 1 A photograph of Radix Puerariae Thomsonii

1. NAMES

Official Name: Radix Puerariae Thomsonii

Chinese Name: 粉葛

Chinese Phonetic Name: Fenge

2. SOURCE

Radix Puerariae Thomsonii is the dried root of *Pueraria thomsonii* Benth. (Fabaceae). The root is collected in autumn or winter, washed clean, the outer bark removed, dried slightly and cut into oblique thick slices or small cubes, then fully dried under the sun to obtain Radix Puerariae Thomsonii.

3. DESCRIPTION

Intact roots cylindrical to subfusiform and conical, 0.4-39.3 cm in length, 27-84 mm in diameter; cut slices are oblique or transversely oriented or in the form of small cubes, variable in size. Externally yellowish-white, with remnant outer bark pale-brown. Texture heavy, hard, and starchy; transverse section pale yellowish-brown, appearing as concentric annular rings composed of fibres, the colour distinctly pale brown. Odourless; taste slightly sweetish (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Residual cork broad, consisting of several rows of densely arranged cork cells. Cortex narrow. Fibre bundles are arranged alternately and aligned with the vessels, surrounded by parenchyma cells containing prisms of calcium oxalate, which appear as crystal fibres. Phloem and xylem are arranged alternately into hetero-vascular bundles, forming 3-5 concentric rings; relatively large vessels in the xylem densely packed and alternately disposed with fibre bundles; rays narrow, parenchyma cells filled with starch granules (Fig. 2).

Powder

Colour yellowish-white. Numerous starch granules, mostly of the compound type, and a few simple ones, are observed; simple granules spheroidal, subglobose, 4-38 µm in diameter, with dotted, cleft or asteroïdal hilum; compound granules composed of 2-15 simple granules, 8-78 µm in diameter. Fibres mostly in bundles, with thickened walls, surrounded by parenchyma cells containing prisms of calcium oxalate. Vessels mostly bordered-pitted (Fig. 3).

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

Standard solution

Puerarin standard solution

Weigh 1.0 mg of puerarin CRS (Fig. 4) and dissolve in 1 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and water (12:3:1, v/v).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (90 W) the mixture for 10 min. Filter through a filter paper.

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 puerarin standard solution (1 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 (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 puerarin.

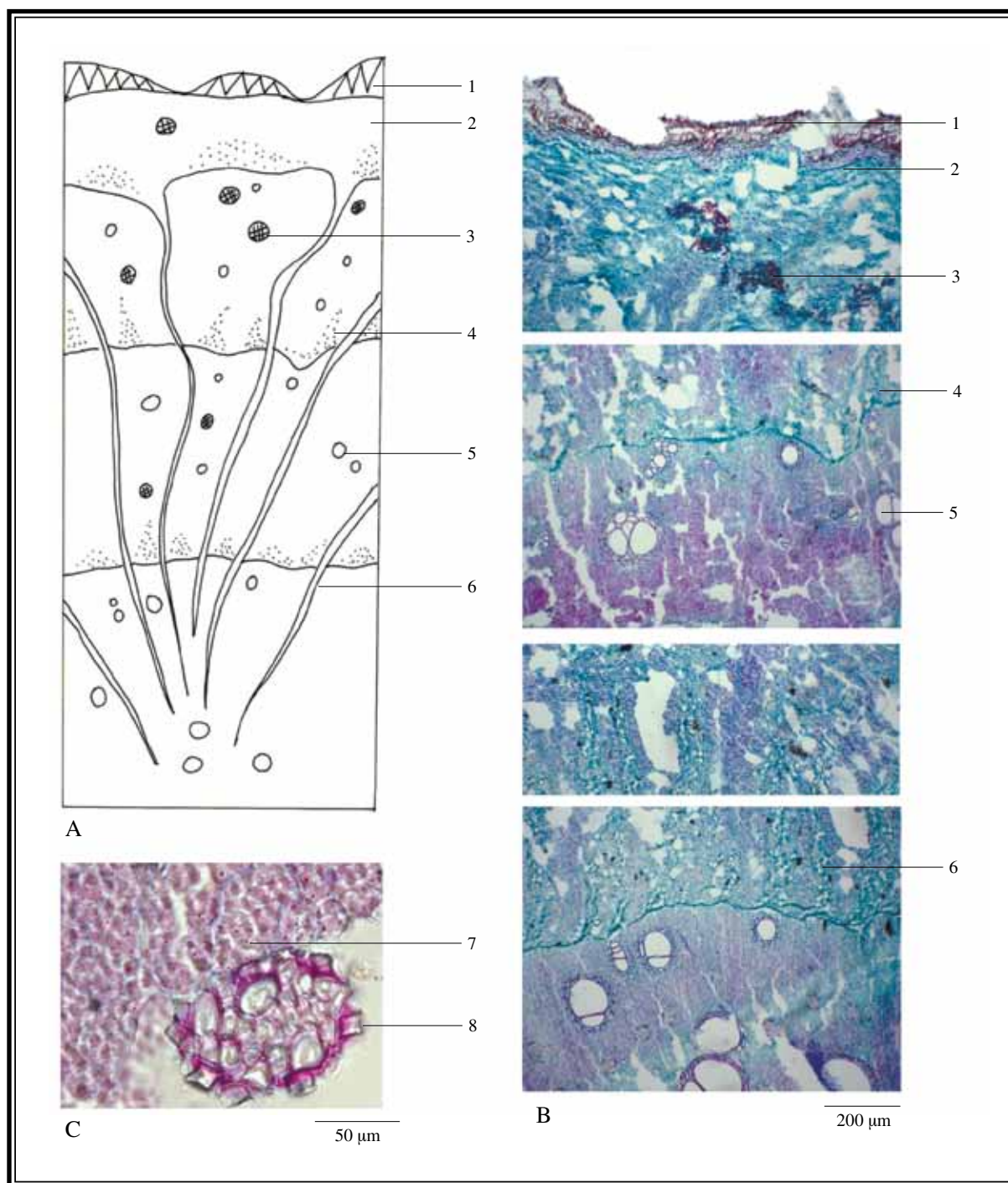


Figure 2 Microscopic features of transverse section of *Radix Puerariae Thomsonii*

A. Sketch B. Section illustration C. Fibre bundle

1. Cork
2. Cortex
3. Fibre bundle
4. Phloem
5. Xylem
6. Xylem ray
7. Starch granules
8. Prism of calcium oxalate

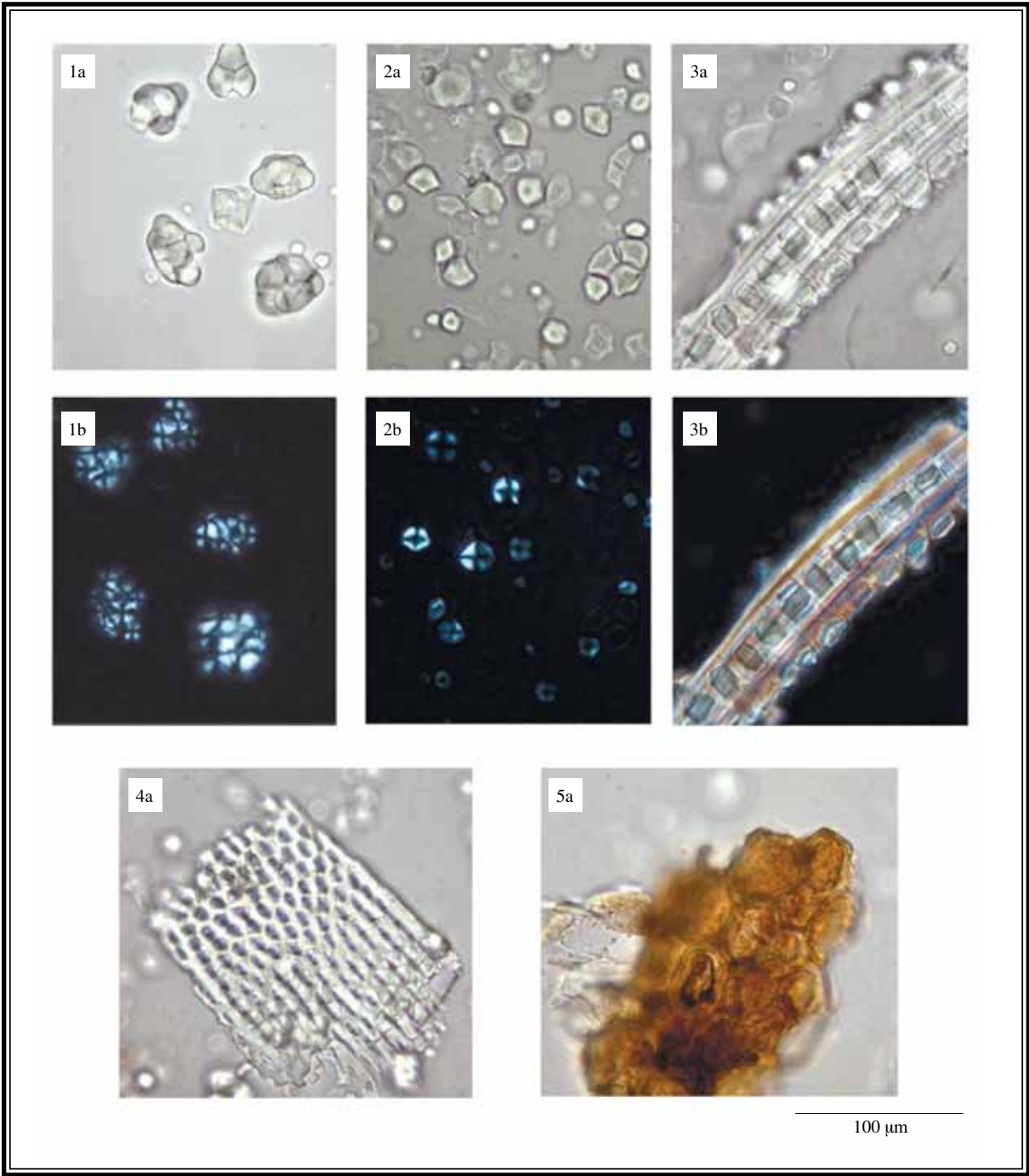


Figure 3 Microscopic features of powder of *Radix Puerariae Thomsonii*

1. Compound starch granules
 2. Simple starch granules
 3. Crystal fibres
 4. Vessels
 5. Cork cells
- a. Features under the light microscope b. Features under the polarized microscope

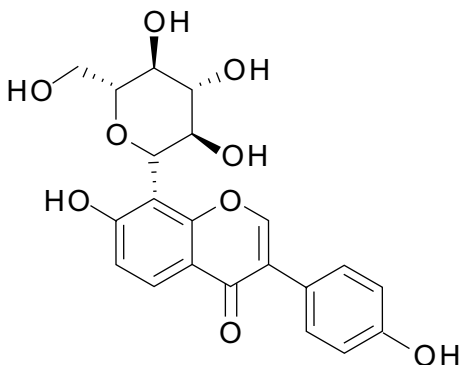


Figure 4 Chemical structure of puerarin

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

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

Weigh 1.0 mg of puerarin CRS and dissolve in 10 mL of ethanol (70 %).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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)	0.1% Formic acid (% , v/v)	Acetonitrile (% , v/v)	Elution
0–40	90→65	10→35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of puerarin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of puerarin should not be more than 5.0%; the RSD of the retention time of puerarin peak should not be more than 2.0%; the column efficiency determined from puerarin peak should not be less than 20000 theoretical plates.

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

Procedure

Separately inject puerarin Std-FP and the test solution (5 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of puerarin peak in the chromatogram of puerarin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify puerarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of puerarin Std-FP. The retention times of puerarin 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 Radix Puerariae Thomsonii extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1 (marker, puerarin)	1.00	-
2	1.06	± 0.03
3 (daidzin)	1.33	± 0.03
4	2.54	± 0.06

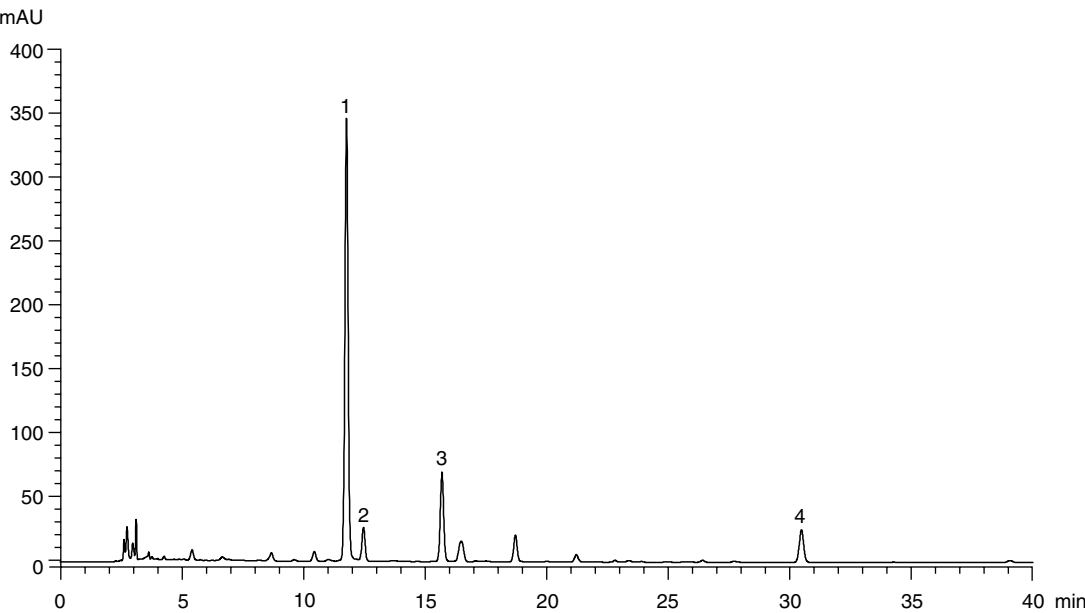


Figure 5 A reference fingerprint chromatogram of Radix Puerariae Thomsonii extract

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 – Aflatoxins** (*Appendix VII*): meet the requirements.
- 5.4 Sulphur Dioxide Residues** (*Appendix XV*): not more than 400 mg/kg.
- 5.5 Foreign Matter** (*Appendix VIII*): not more than 1.0%.
- 5.6 Ash** (*Appendix IX*)
- Total ash: not more than 4.0%.
- Acid-insoluble ash: not more than 0.5%.
- 5.7 Water Content** (*Appendix X*): not more than 11.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 57.0%.

Ethanol-soluble extractives (hot extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

Puerarin standard stock solution, Std-Stock (800 mg/L)

Weigh accurately 8.0 mg of puerarin CRS and dissolve in 10 mL of ethanol (30%).

Puerarin standard solution for assay, Std-AS

Measure accurately the volume of the puerarin Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 16, 40, 80, 160, 240 mg/L for puerarin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (70%). Sonicate (90 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the extracts and make up to the mark with ethanol (70%). Mix and filter through a 0.45- μ m RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (250 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. The mobile phase is a mixture of acetonitrile and water (10:90, v/v). The elution time is about 25 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of puerarin Std-AS (80 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of puerarin should not be more than 5.0%; the RSD of the retention time of puerarin peak should not be more than 2.0%; the column efficiency determined from puerarin peak should not be less than 8000 theoretical plates.

The *R* value between puerarin 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 puerarin Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of puerarin against the corresponding concentrations of puerarin Std-AS. Obtain the slope, y-intercept and the *r*² value from the 5-point calibration curve.

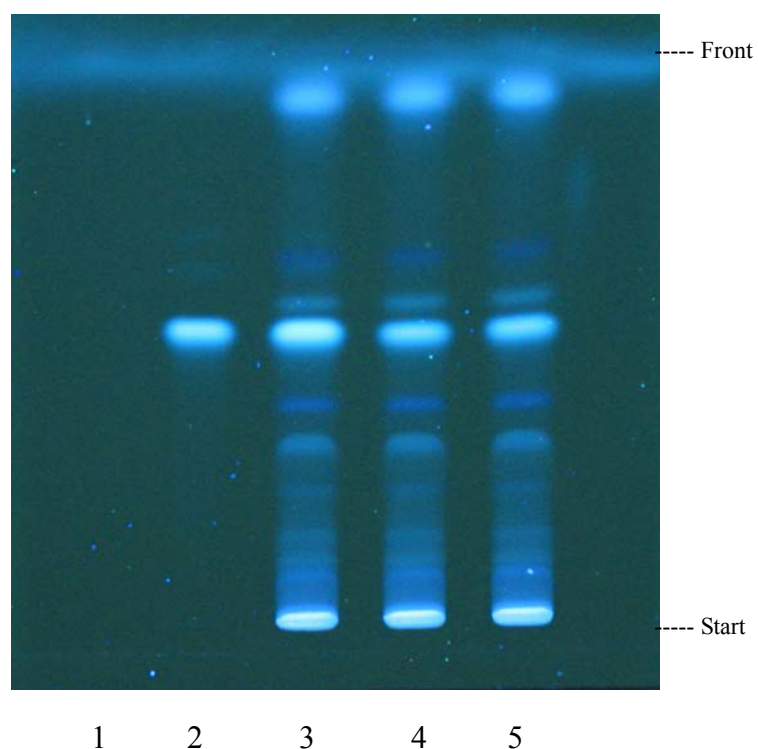
Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify puerarin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of puerarin Std-AS. The retention times of puerarin 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 puerarin in the test solution, and calculate the percentage content of puerarin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.16% of puerarin (C₂₁H₂₀O₉), calculated with reference to the dried substance.

Radix Puerariae Thomsonii (粉葛)



Lane	Sample	Results
1	Blank (70% ethanol)	Negative
2	Standard (Puerarin)	Puerarin positive
3	Spiked sample (Sample plus puerarin)	Puerarin positive
4	Sample (Radix Puerariae Thomsonii)	Puerarin positive
5	Sample duplicate (Radix Puerariae Thomsonii)	Puerarin positive

Figure 1 TLC results of Radix Puerariae Thomsonii extract observed under UV light (366 nm)