Fructus Psoraleae

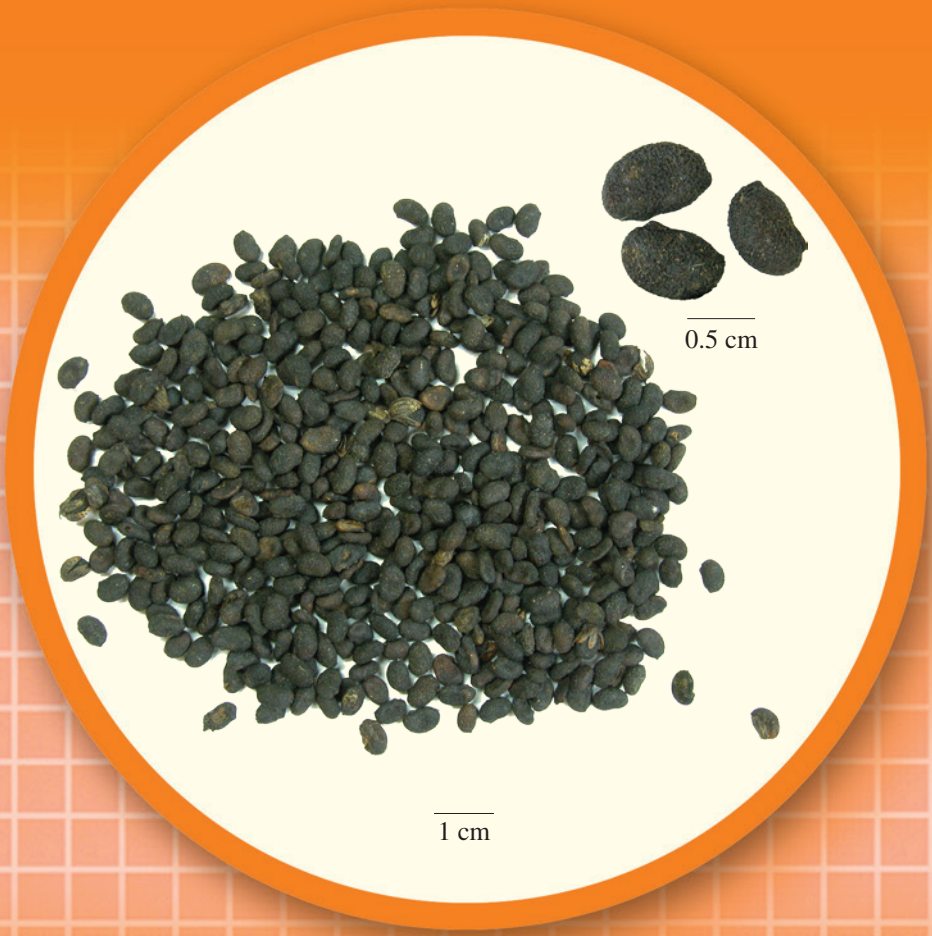


Figure 1 A photograph of Fructus Psoraleae

1. NAMES

Official Name: Fructus Psoraleae

Chinese Name: 補骨脂

Chinese Phonetic Name: Buguzhi

2. SOURCE

Fructus Psoraleae is the dried ripe fruit of *Psoralea corylifolia* L. (Fabaceae). The fruit is collected in autumn when it is ripe, dried under the sun, and foreign matter removed to obtain Fructus Psoraleae.

3. DESCRIPTION

Ovoid to ellipsoid or reniform, slightly flattened, 3-5 mm long, 2-4 mm wide, about 1-1.5 mm thick. Externally blackish-brown to greyish-brown, with finely reticulate wrinkles. Apex obtuse-rounded, slightly prominent, the depressed end showing a scar of fruit stalk. Texture hard. Pericarp thin, not easily stripped from the seed; seed 1, cotyledons 2, yellowish-white, oily. Odour aromatic; taste pungent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of epidermal cells. Intramural glands arranged under the epidermal cells of the exocarp and often broken off. Mesocarp consists of small vascular bundles and mesocarp parenchyma cells; some mesocarp cells contain minute crystals of calcium oxalate. Epidermal cells of testa consist of 1 layer of 35-63 μm long cylindrical palisade cells and 1 layer of sunken support cells, and below it, several layers of shrunken or compressed parenchyma cells make up the hypodermal tissue. Next to the hypodermis is the endodermal tissue of the testa, consisting of 1 layer of elongated-oblong to suborbicular pigmented parenchyma cells. The cotyledons consist of parenchyma cells containing small aleurone and fatty oil, while the endosperm and radicle consist of simple parenchyma cells (Fig. 2).

Powder

Colour greyish-yellow. Cylindrical palisade cells of testa yellowish-brown, 35-63 μm long, cell wall thickened, appearing as a “V” shape in lateral view, subpolygonal in surface view. Crystals of calcium oxalate minute, scattered in the mesocarp parenchyma cells, polychromatic

under a polarized microscope. Unicellular non-glandular hair present, 150-568 μm in length, with densely dotted protuberances on the surface. Support cells of testa sunken in lateral view, thicker cell wall in the middle. Glandular hairs capped by a multicellular or unicellular head with a short stalk. Intramural glands round when completely intact, 160-400 μm in diameter, but more often broken into pieces; the cells in the middle are smaller, polygonal in shape, and surrounded by radially oriented elongated cells (Fig. 3).

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

Standard solutions

Isopsoralen standard solution

Weigh 1.0 mg of isopsoralen CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Psoralen standard solution

Weigh 1.0 mg of psoralen CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and acetic acid (3:1:0.05, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of water. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Concentrate the solution to about 10 mL at about 60°C at reduced pressure in a rotary evaporator. Transfer the solution into a 50-mL centrifuge tube. Mix the solution with 10 mL of ethyl acetate for about 1 min. Allow to stand for about 5 min until the two layers are separated. Transfer the ethyl acetate extract to a 100-mL round-bottomed flask. Repeat the extraction for one more time. Combine the ethyl acetate extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately isopsoralen standard solution, psoralen standard solution (3 μL each) and the test solution (2 μL) to the plate. Develop over a path of about 7.5 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).

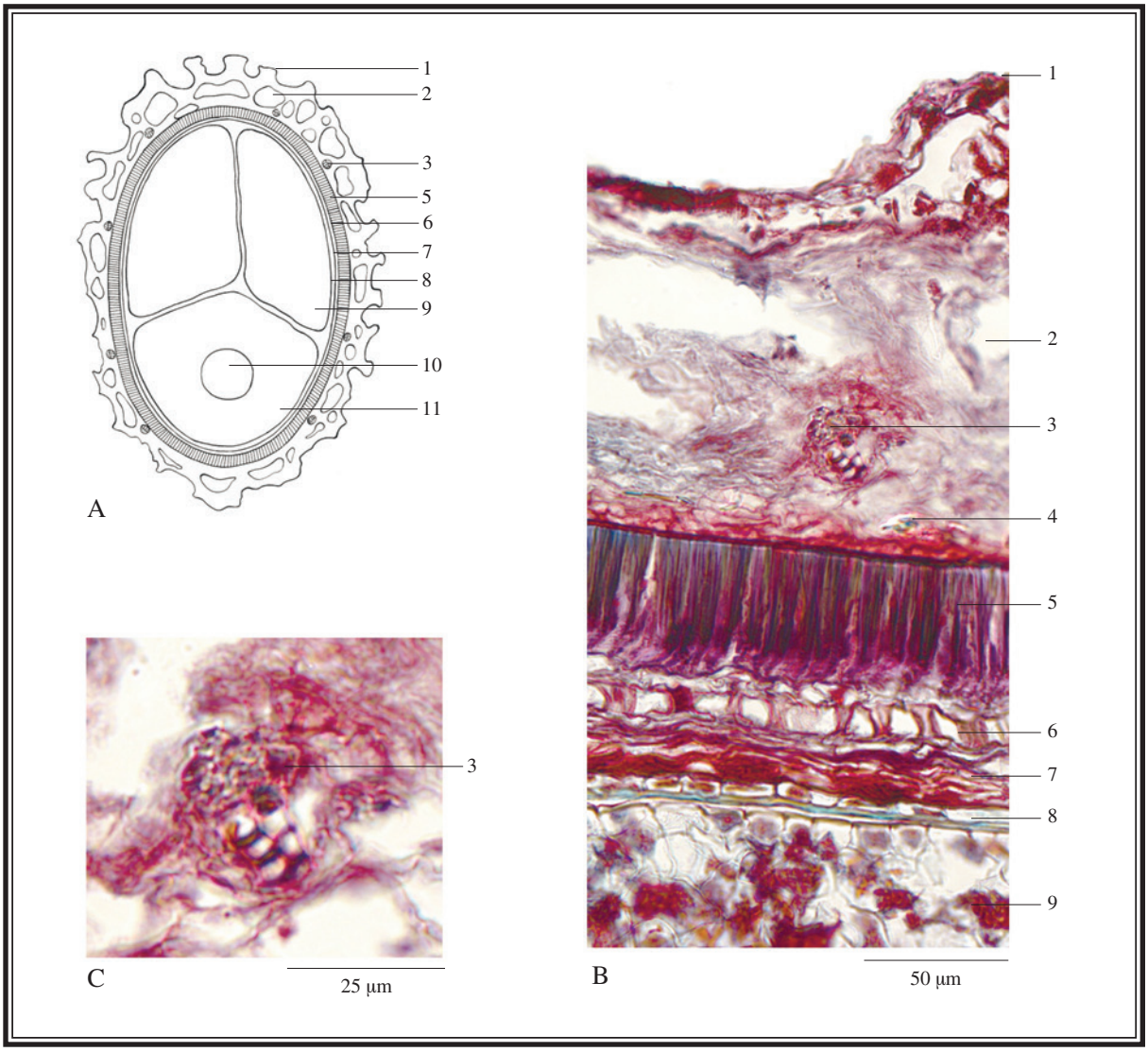


Figure 2 Microscopic features of transverse section of Fructus Psoraleae

A. Sketch B. Section illustration C. Vascular bundle

1. Exocarp 2. Intramural gland 3. Vascular bundle 4. Crystal of calcium oxalate
5. Cylindrical palisade cells of testa 6. Support cells of testa 7. Hypodermal cells of testa
8. Endospermal cells of testa 9. Cotyledon 10. Radicle 11. Endosperm

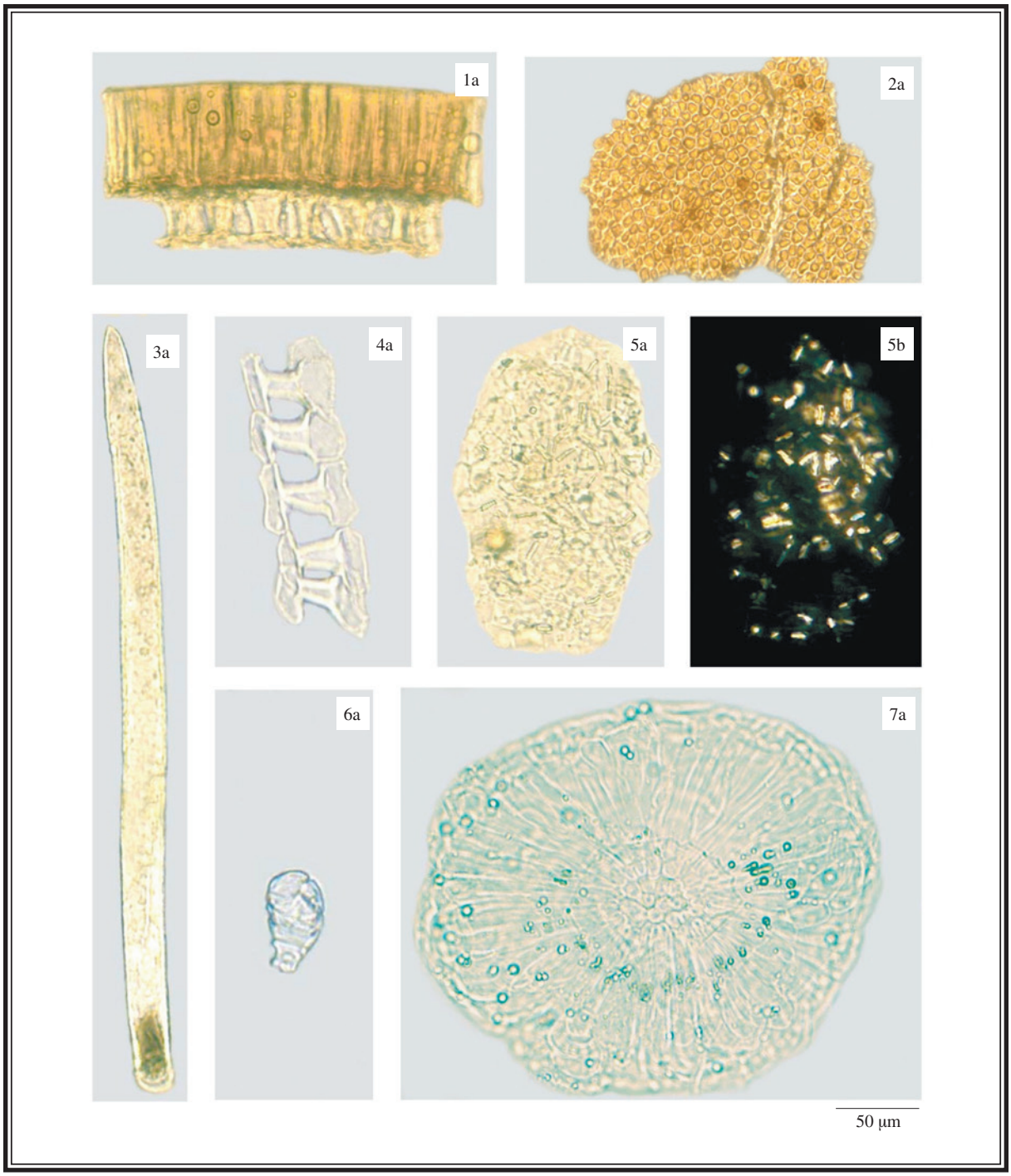


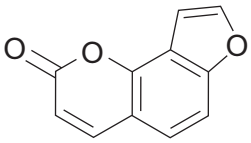
Figure 3 Microscopic features of powder of Fructus Psoraleae

1. Cylindrical palisade cells of testa (Lateral view)
2. Cylindrical palisade cells of testa (Surface view)
3. Unicellular non-glandular hairs 4. Support cells of testa
5. Crystals of calcium oxalate 6. Glandular hair 7. Intramural glands

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

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 isopsoralen and psoralen.

(i)



(ii)

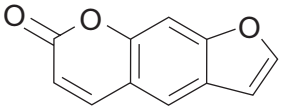


Figure 4 Chemical structures of (i) isopsoralen and (ii) psoralen

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

Standard solution

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

Weigh 1.0 mg of isopsoralen CRS and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 5 min. Filter the supernatant through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (308 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0–20	50→30	50→70	linear gradient
20–45	30→15	70→85	linear gradient
45–50	15→10	85→90	linear gradient
50–60	10	90	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of isopsoralen Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isopsoralen should not be more than 5.0%; the RSD of the retention time of isopsoralen peak should not be more than 2.0%; the column efficiency determined from isopsoralen peak should not be less than 6000 theoretical plates.

The *R* value between peak 1 and peak 2 in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject isopsoralen Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of isopsoralen peak in the chromatogram of isopsoralen Std-FP and the retention times of the eight characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify isopsoralen peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isopsoralen Std-FP. The retention times of isopsoralen 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 eight characteristic peaks of Fructus Psoraleae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the eight characteristic peaks of Fructus Psoraleae extract

Peak No.	RRT	Acceptable Range
1 (psoralen)	0.92	±0.03
2 (marker, isopsoralen)	1.00	-
3	2.01	±0.04
4	2.15	±0.03
5	2.73	±0.03
6	3.06	±0.03
7	3.30	±0.04
8	3.73	±0.04

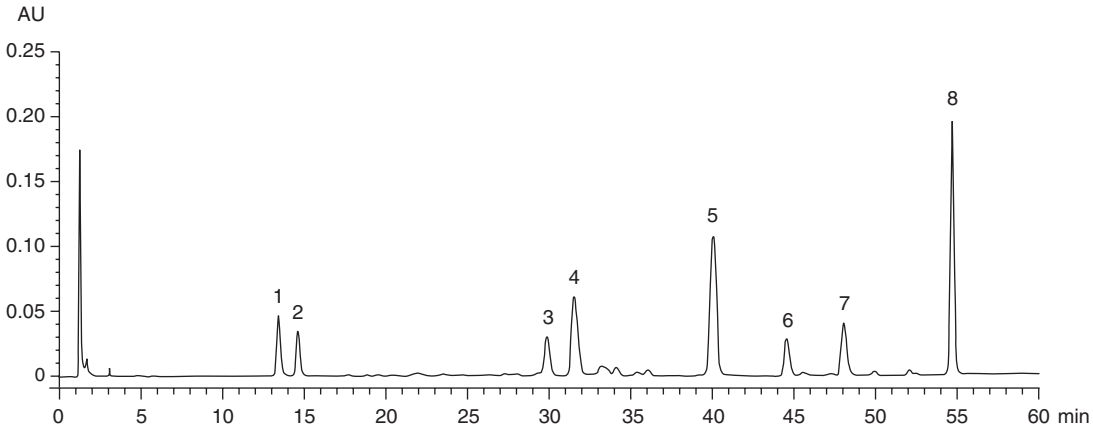


Figure 5 A reference fingerprint chromatogram of Fructus Psoraleae extract

For positive identification, the sample must give the above eight 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)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 20.0%.
Ethanol-soluble extractives (cold extraction method): not less than 23.0%.

7. ASSAY

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

Standard solution

Mixed psoralen and isopsoralen standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of psoralen CRS and 2.0 mg of isopsoralen CRS, and dissolve in 10 mL of methanol.

Mixed psoralen and isopsoralen standard solution for assay, Std-AS

Measure accurately the volume of the mixed psoralen and isopsoralen Std-Stock, dilute with methanol to produce a series of solutions of 10, 40, 60, 100, 150 mg/L for both psoralen and isopsoralen.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 2000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatant. Make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (246 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 methanol and water (50:50, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed psoralen and isopsoralen Std-AS (60 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of psoralen and isopsoralen should not be more than 5.0%; the RSD of the retention times of psoralen and isopsoralen peaks should not be more than 2.0%; the column efficiencies determined from psoralen and isopsoralen peaks should not be less than 3500 and 4000 theoretical plates respectively.

The *R* value between psoralen peak and isopsoralen peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed psoralen and isopsoralen Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of psoralen and isopsoralen against the corresponding concentrations of the mixed psoralen and isopsoralen Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

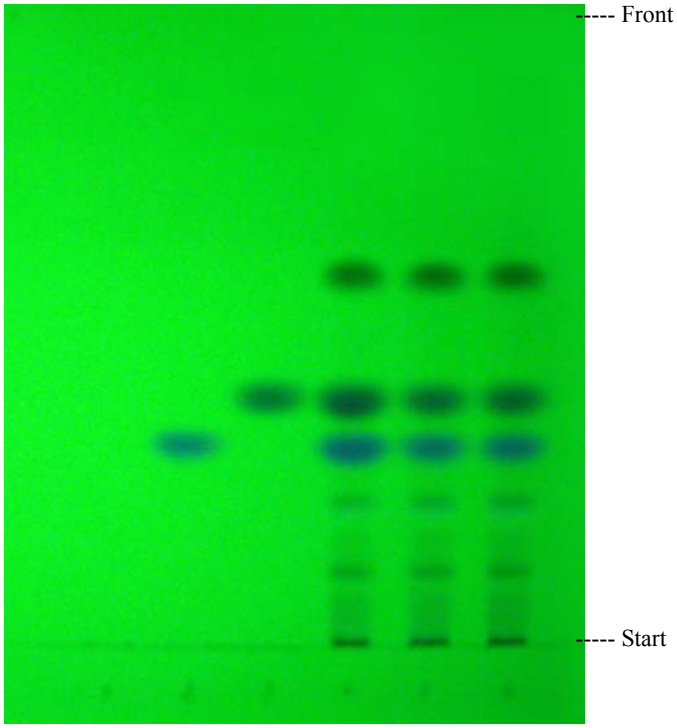
Procedure

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify psoralen and isopsoralen peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed psoralen and isopsoralen Std-AS. The retention times of psoralen and isopsoralen peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of psoralen and isopsoralen in the test solution, and calculate the percentage contents of psoralen and isopsoralen in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 1.6% of the total content of psoralen ($C_{11}H_6O_3$) and isopsoralen ($C_{11}H_6O_3$), calculated with reference to the dried substance.

Fructus Psoraleae (補骨脂)



Lane	Sample	Results
1	Blank (Ethyl acetate)	Negative
2	Standard (Psoralen)	Psoralen positive
3	Standard (Isopsoralen)	Isopsoralen positive
4	Spiked sample (Sample plus psoralen and isopsoralen)	Psoralen and isopsoralen positive
5	Sample (Fructus Psoraleae)	Psoralen and isopsoralen positive
6	Sample duplicate (Fructus Psoraleae)	Psoralen and isopsoralen positive

Figure 1 TLC results of Fructus Psoraleae extract observed under UV light (254 nm)