Sophorae Fructus

Figure 1 A photograph of Sophorae Fructus

A. Sophorae Fructus B. Seeds and cotyledons

1. NAMES

Official Name: Sophorae Fructus

Chinese Name: 槐角

Chinese Phonetic Name: Huaijiao

2. SOURCE

Sophorae Fructus is the dried ripe fruit of *Sophora japonica* L. (Fabaceae). The ripe fruit is collected in winter, foreign matter removed, then dried under the sun to obtain Sophorae Fructus.

3. DESCRIPTION

Beaded, 1-8 cm long, 6-10 mm in diameter. Externally yellowish-green to yellowish-brown, shrunken and rough, the side of one dorsal suture yellow. Texture soft, shrunken after drying, easily broken at the collapsed part; fracture yellowish-green, viscous. Seeds 1-6, reniform, 6-11 mm long, surface smooth, black to brownish-black; hilum at the concave side round, greyish-white; texture hard, cotyledons 2, yellow to yellowish-green. Odour of pericarp slight; taste bitter. Seeds taste bean-like on chewing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exocarp consists of 1 layer of rectangular epidermal cells, covered with cuticle. Mesocarp consists of parenchymatous cells in various layers, occasionally more than 30 layers, scattered with prisms of calcium oxalate. The cells of the outer layers and inner layers of mesocarp arranged closely, parenchymatous cells in the middle part relatively large, with small groups of stone cells and vascular bundles found scattered opposite the seed hilum. Endocarp consists of 1 layer of small subrectangular parenchymatous cells, prolonged tangentially. Testa consists of 1 layer of palisade cells in outer side, with strongly lignified wall, beneath showing 1 layer of beaker cells, and several layers of parenchymatous cells in inner side. Oval tracheid island located inside the cavity of the seed hilum, consisting of many scalariform and reticulate tracheids. Stellate tissue present in both sides of the tracheid island. Cotyledon surrounded by endosperm cells (Fig. 2).

Powder

Colour greyish-brown to brown. Epidermal cells of exocarp polygonal in the surface view, wall slightly thickened, stomata actinocytic visible. Mesocarp cells elliptical or elongated. Prisms of calcium oxalate usually present in mesocarp and parenchymatous cells, diamond-shaped or columnar, 4-24 μm in diameter. Palisade cells of testa colourless to brown, columnar and neatly arranged in lateral view, subrounded in bottom or surface view. Beaker cells of testa dumbbell-shaped and subrectangular in lateral view, subrounded in surface view. Stone cells yellow to greenish-yellow, varied in shape, subrectangular, subrounded, triangular or shell-shaped. Cotyledon cells contain oil droplets (Fig. 3).

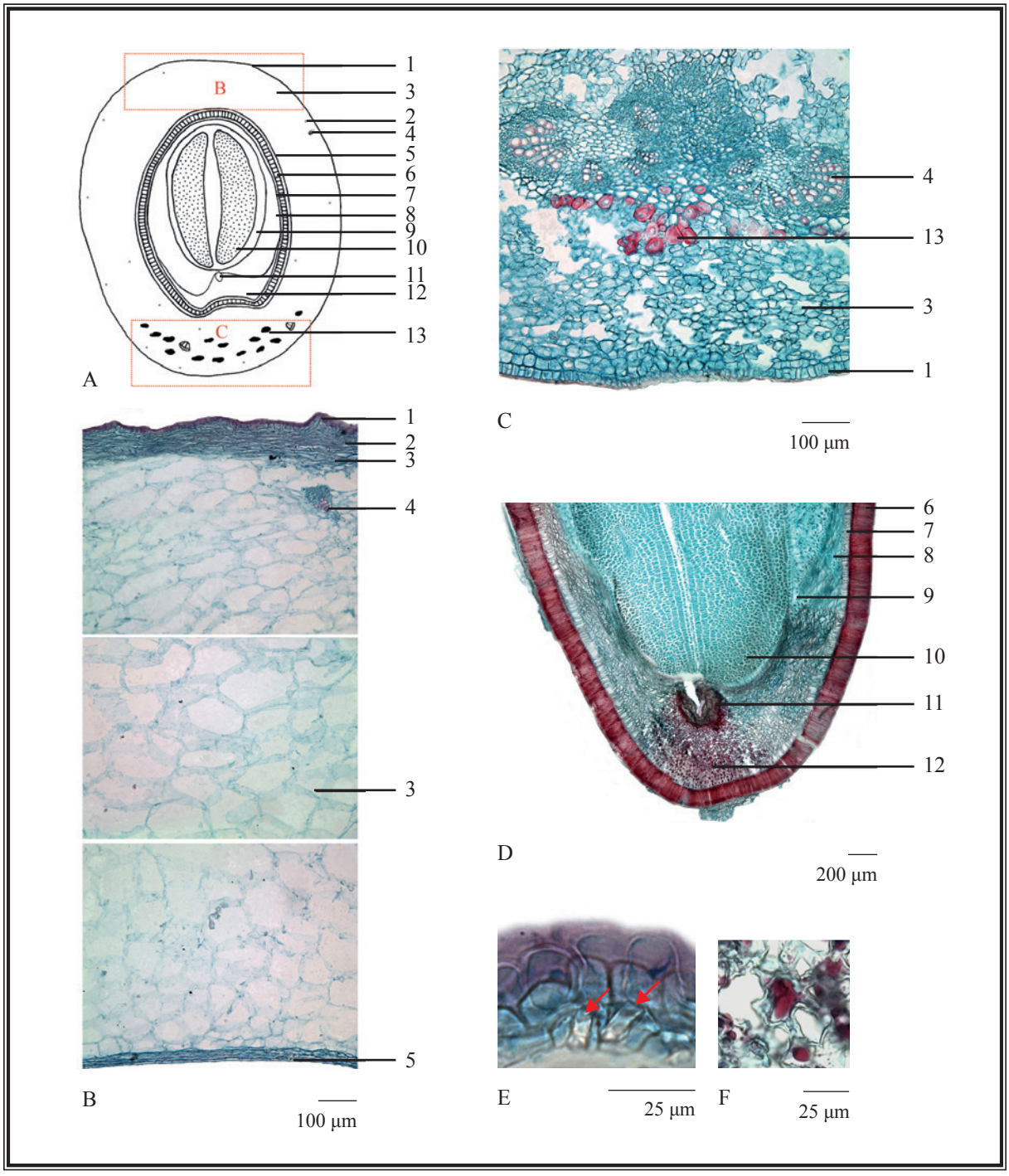


Figure 2 Microscopic features of transverse section of Sophorae Fructus

A. Sketch B. Pericarp section illustration C. Pericarp section (near seed hilum) illustration
D. Seed section illustration E. Prisms of calcium oxalate F. Stellate tissue

1. Exocarp 2. Prisms of calcium oxalate 3. Mesocarp 4. Vascular bundle
5. Endocarp 6. Testa 7. Beaker cells 8. Parenchyma of testa
9. Endosperm 10. Cotyledon 11. Tracheid island 12. Stellate tissue 13. Stone cells

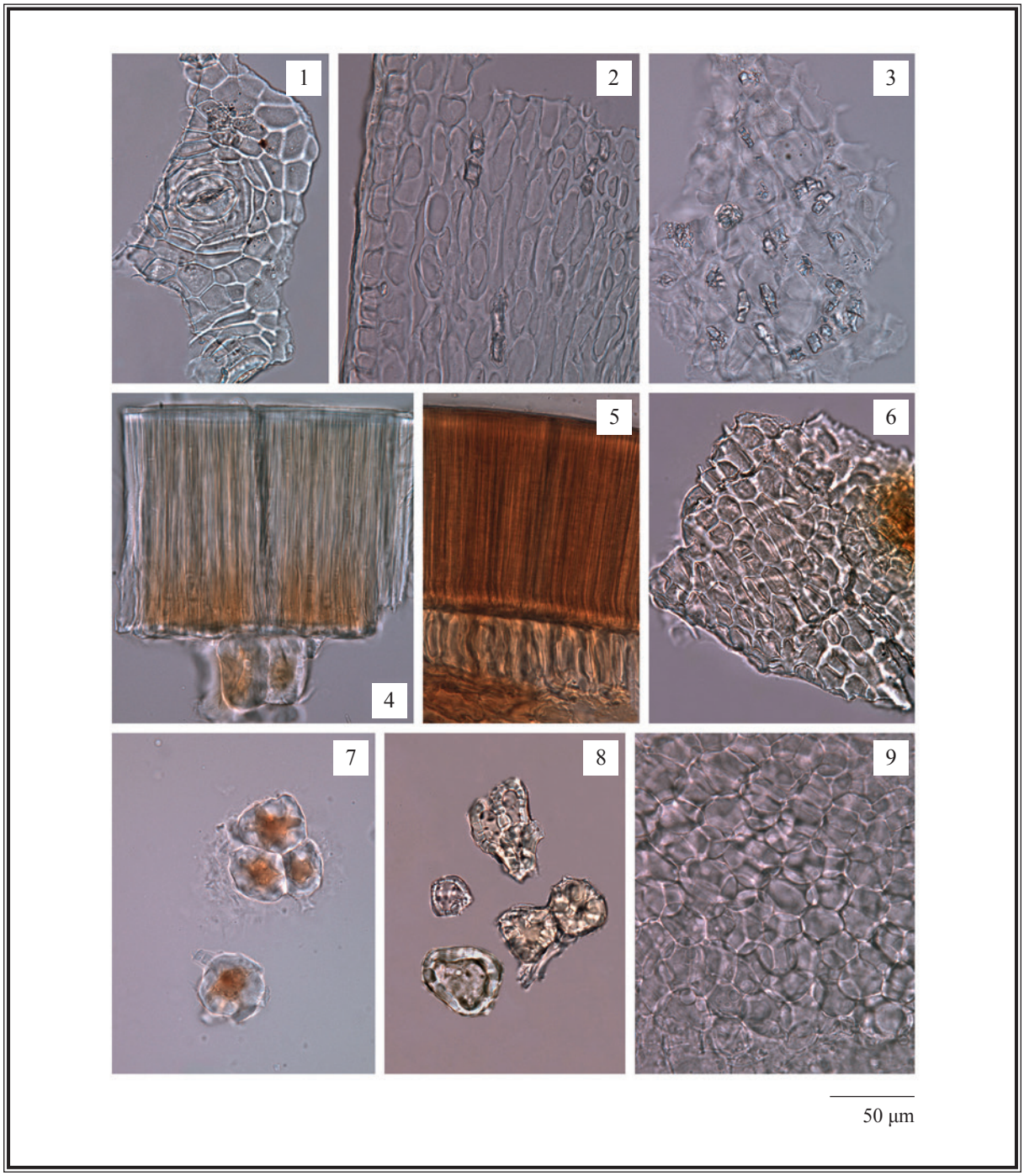


Figure 3 Microscopic features of powder of Sophorae Fructus (under the light microscope)

1. Exocarp with an actinocytic type stoma
2. Mesocarp with prisms of calcium oxalate 3. Prisms of calcium oxalate in parenchymatous cells
4. Palisade cells and beaker cells of testa (in lateral view)
5. Palisade cells, beaker cells and parenchymatous cells of testa (in lateral view)
6. Palisade cells of testa (in surface view)
7. Beaker cells (in surface view) 8. Stone cells 9. Cotyledon cells

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

Standard solution

Sophoricoside standard solution

Weigh 1.0 mg of sophoricoside CRS (Fig. 4) and dissolve in 2 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol and acetic acid (7.5:1:0.5, v/v).

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (160 W) the mixture for 30 min. Filter the mixture.

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 sophoricoside standard solution and the test solution (3 μL each) to the plate. Develop over a path of about 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 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 sophoricoside.

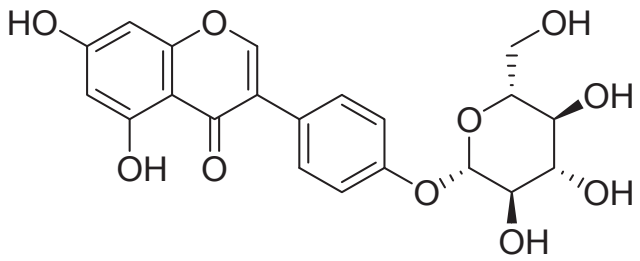


Figure 4 Chemical structure of sophoricoside

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

Standard solution

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

Weigh 0.4 mg of sophoricoside CRS and dissolve in 20 mL of ethanol (70%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (70%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (70%). Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 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)	0.4% Formic acid (%, v/v)	Acetonitrile:Methanol (50:50, v/v) (%, v/v)	Elution
0 – 60	85 → 70	15 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of sophoricoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of sophoricoside should not be more than 5.0%; the RSD of the retention time of sophoricoside peak should not be more than 2.0%; the column efficiency determined from sophoricoside peak should not be less than 30000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject sophoricoside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of sophoricoside peak in the chromatogram of sophoricoside Std-FP and the retention times of the five characteristic

peaks (Fig. 5) in the chromatogram of the test solution. Identify sophoricoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sophoricoside Std-FP. The retention times of sophoricoside 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 five characteristic peaks of Sophorae Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Sophorae Fructus extract

Peak No.	RRT	Acceptable Range
1	0.62	± 0.03
2	0.70	± 0.03
3 (rutin)	0.81	± 0.03
4 (marker, sophoricoside)	1.00	-
5	1.07	± 0.03

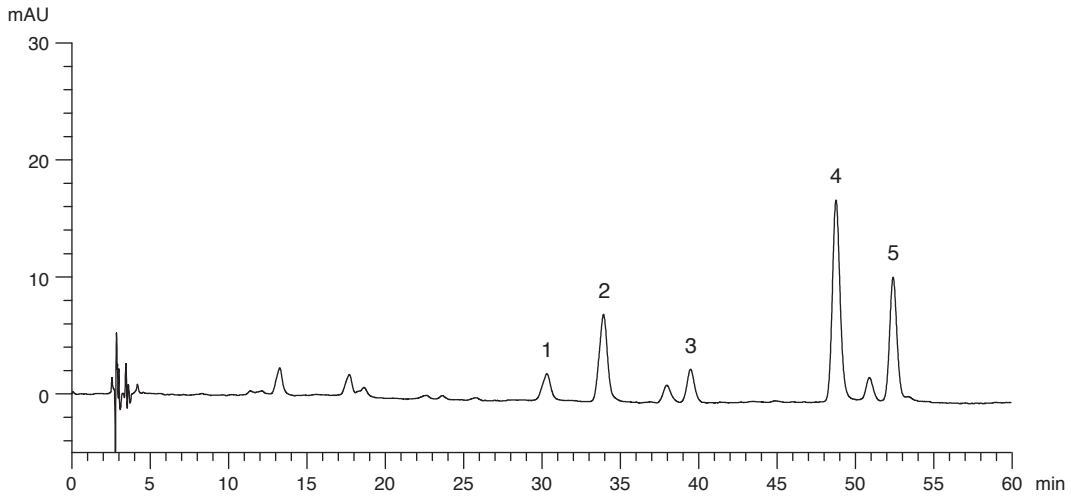


Figure 5 A reference fingerprint chromatogram of Sophorae Fructus extract

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

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 43.0%.
Ethanol-soluble extractives (cold extraction method): not less than 45.0%.

7. ASSAY

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

Standard solution

Sophoricoside standard stock solution, Std-Stock (100 mg/L)

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

Sophoricoside standard solution for assay, Std-AS

Measure accurately the volume of the sophoricoside Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 0.5, 10, 20, 30, 40 mg/L for sophoricoside.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (70%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (70%). Pipette 1 mL of the solution to a 10-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.4% Formic acid (%, v/v)	Acetonitrile:Methanol (50:50, v/v) (%, v/v)	Elution
0 – 60	85 ⟶ 70	15 ⟶ 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of sophoricoside Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of sophoricoside should not be more than 5.0%; the RSD of the retention time of sophoricoside peak should not be more than 2.0%; the column efficiency determined from sophoricoside peak should not be less than 30000 theoretical plates.

The *R* value between sophoricoside 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 sophoricoside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of sophoricoside against the corresponding concentrations of sophoricoside Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

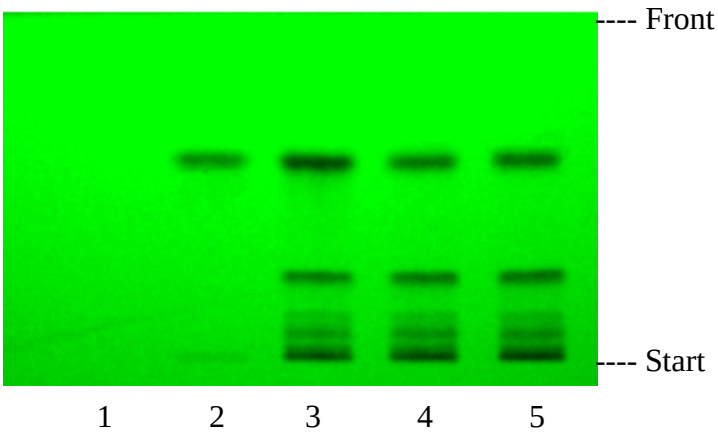
Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify sophoricoside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sophoricoside Std-AS. The retention times of sophoricoside 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 sophoricoside in the test solution, and calculate the percentage content of sophoricoside in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 4.0% of sophoricoside ($C_{21}H_{20}O_{10}$), calculated with reference to the dried substance.

Sophorae Fructus (槐角)



Lane	Sample	Results
1	Blank (70% Ethanol)	Negative
2	Standard (Sophoricoside)	Sophoricoside positive
3	Spiked sample (Sample plus sophoricoside)	Sophoricoside positive
4	Sample (Sophorae Fructus)	Sophoricoside positive
5	Sample duplicate (Sophorae Fructus)	Sophoricoside positive

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