Plantaginis Semen

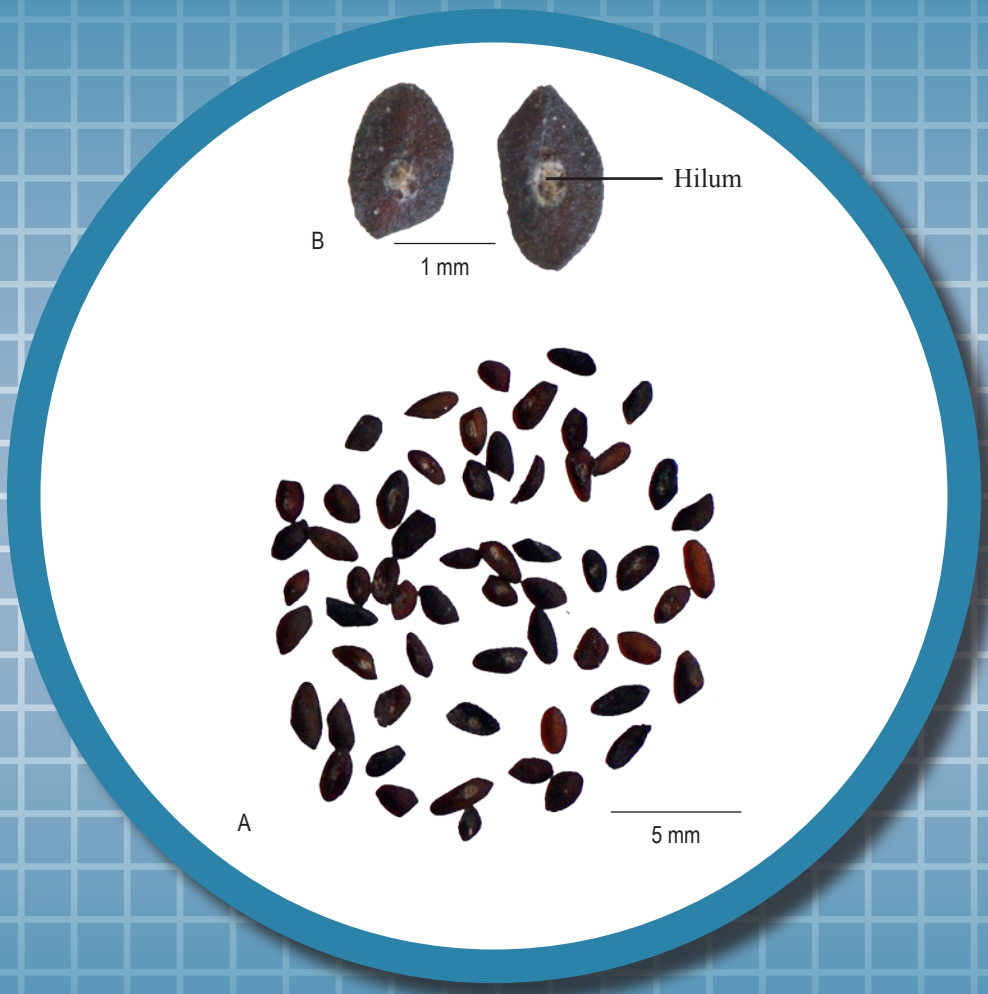


Figure 1 A photograph of Plantaginis Semen

A. Plantaginis Semen B. Magnified seeds

1. NAMES

Official Name: Plantaginis Semen

Chinese Name: 車前子

Chinese Phonetic Name: Cheqianzi

2. SOURCE

Plantaginis Semen is the dried ripe seed of *Plantago asiatica* L. (Plantaginaceae). The fruit-spike is collected in summer and autumn when seed is ripe, and dried the fruit-spike under the sun. The seed gathered and foreign matter removed, then dried again under the sun to obtain Plantaginis Semen.

3. DESCRIPTION

Ovoid, irregularly oblong-ovoid or triangular-ovoid oblong, slightly flattened, about 2 mm long, 1 mm wide. Externally yellowish-brown to blackish-brown, with a pitted, greyish-white hilum on one side. Texture hard. Viscous and slippery when moistened. Odour slight; taste weak (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Testa consists of 2 layers of cells; outer layer is mucilaginous layer, cell wall thin, easily broken; inner layer is pigment layer, the cells contain yellowish-brown contents, subsquare or subrectangular, with slightly curved cell wall. Endosperm cells polygonal, subsquare or subrectangular, the lumen filled with aleurone grains. Cotyledon cells subrounded or subsquare, arranged orderly, containing small aleurone grains and oil droplets (Fig. 2).

Powder

Colour dark yellowish-brown. Inner epidermal cells of testa yellowish-brown, subrectangular, 28-85 μm long, 7-29 μm wide, wall thin, slightly curved. Outer epidermal cells of testa subsquare or slightly elongated tangentially, wall thin. Endosperm cells polygonal, wall thickened, lumen filled with aleurone grains. Cotyledon cells subrounded or subsquare, arranged orderly, composed of parenchymatous cells, containing small aleurone grains and oil droplets (Fig. 3).

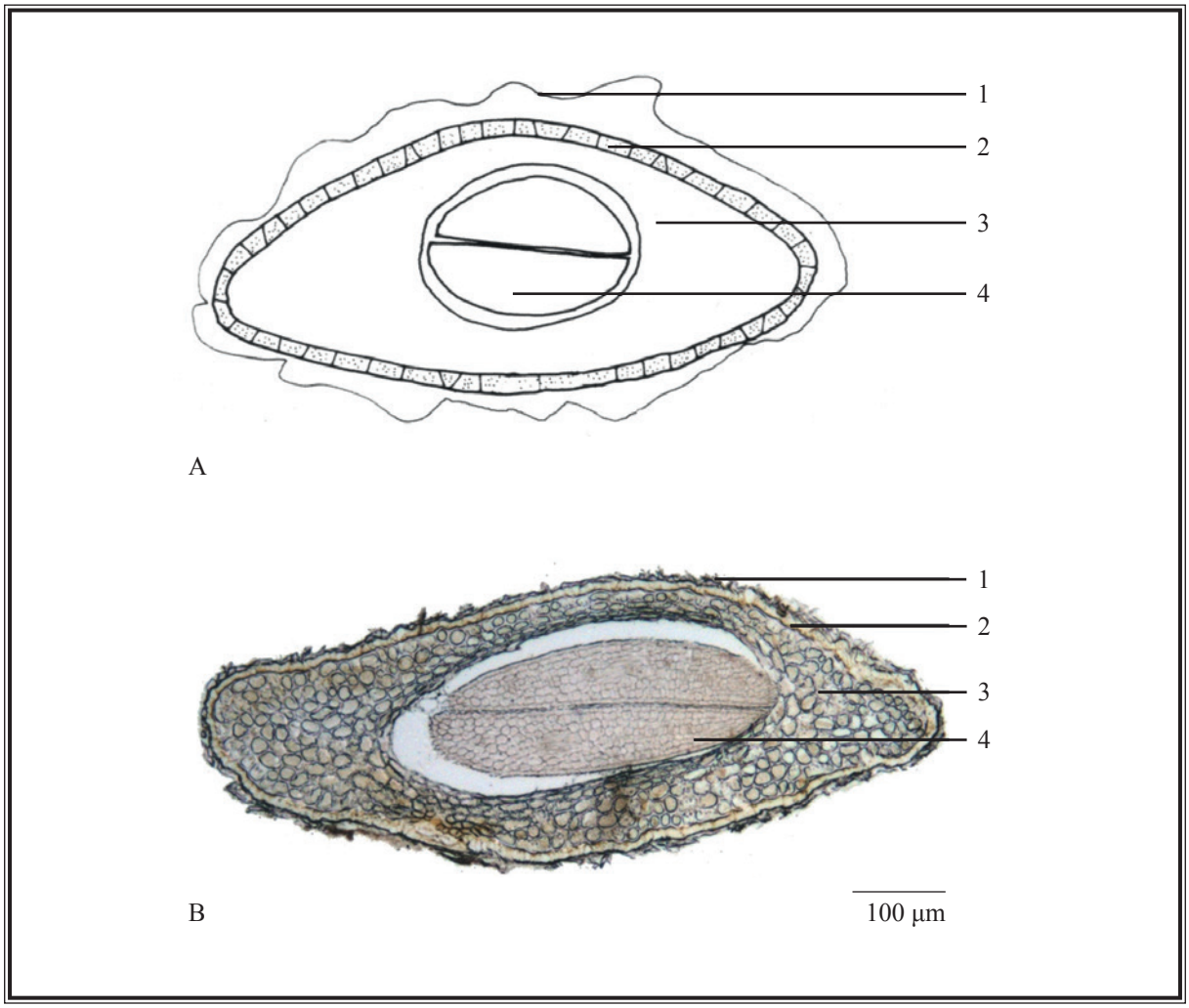


Figure 2 Microscopic features of transverse section of Plantaginis Semen

A. Sketch B. Section illustration

1. Mucilaginous layer (outer layer of testa) 2. Pigment layer (inner layer of testa)
3. Endosperm 4. Cotyledon

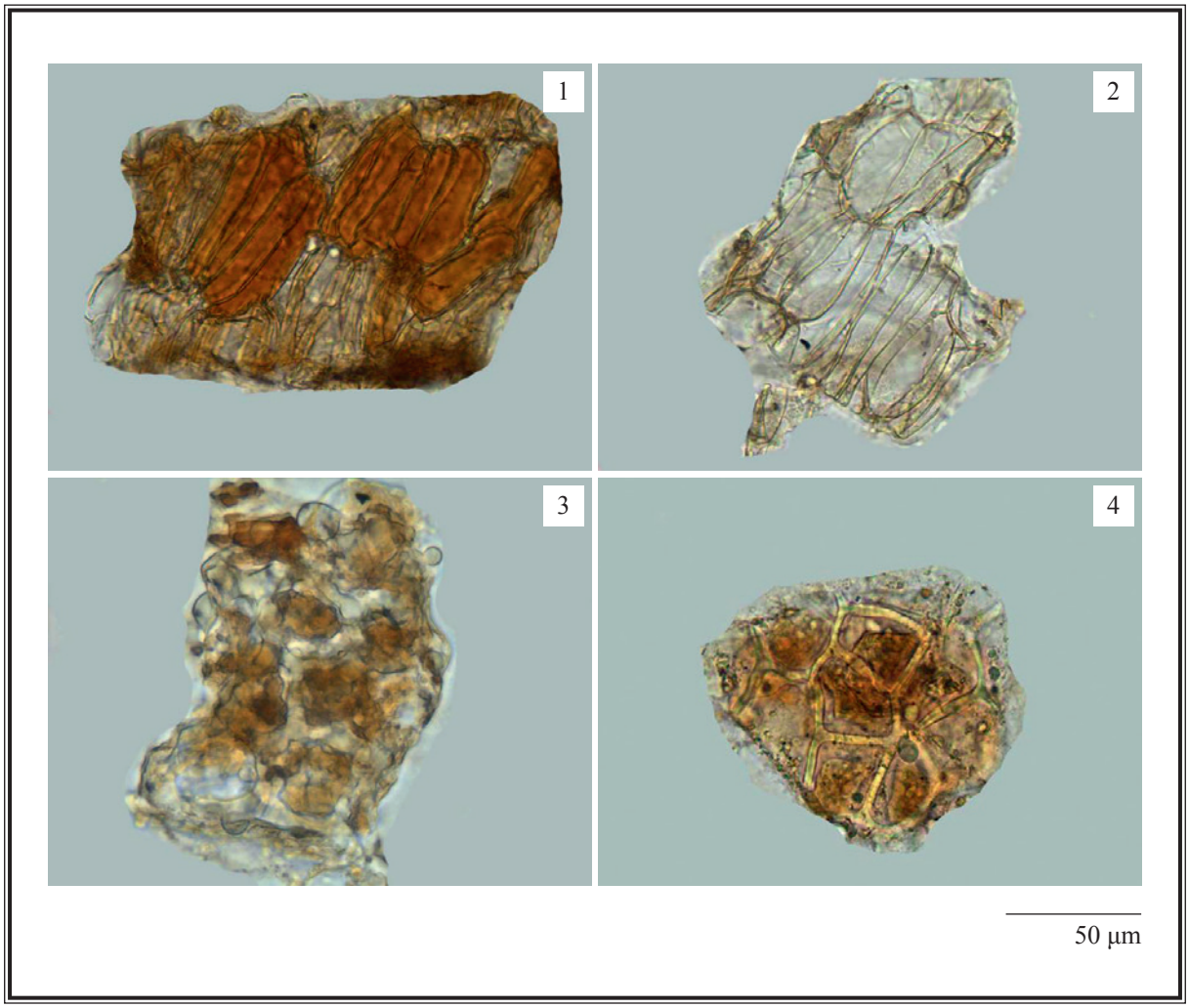


Figure 3 Microscopic features of powder of Plantaginis Semen (under the light microscope)

1. Inner epidermal cells of testa 2. Outer epidermal cells of testa 3. Endosperm cells
4. Cotyledon cells

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

Standard solutions

Acteoside standard solution

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

Geniposidic acid standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, water, formic acid and glacial acetic acid (8:2:1:1, v/v).

Spray reagent

Add slowly 5 mL of sulphuric acid to 95 mL of ethanol and dissolve 1.0 g of vanillin. Freshly prepare the reagent.

Test solution

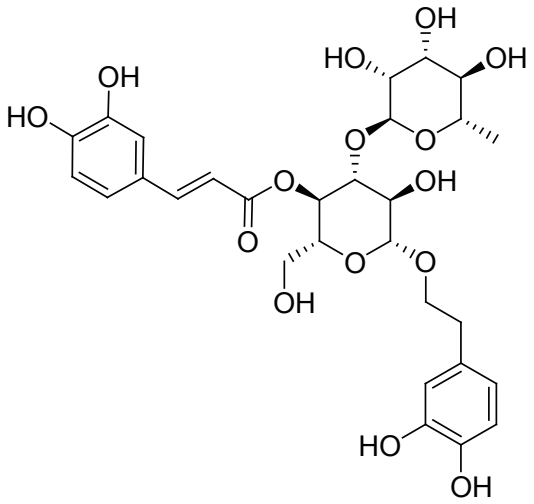
Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (200 W) the mixture for 15 min. Filter the mixture.

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 acteoside standard solution (3 µL), geniposidic acid standard solution (3 µL) and the test solution (4 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 4 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV(A).

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 acteoside and geniposidic acid.

(i)



(ii)

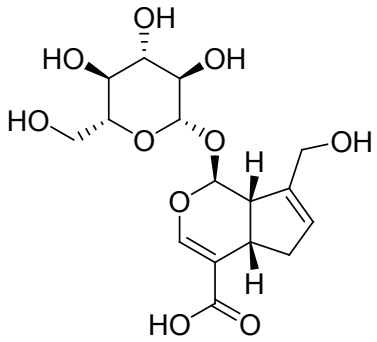


Figure 4 Chemical structures of (i) acteoside and (ii) geniposidic acid

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

Standard solutions

Acteoside standard solution for fingerprinting, Std-FP (680 mg/L)

Weigh 6.8 mg of acteoside CRS and dissolve in 10 mL of methanol.

Geniposidic acid standard solution for fingerprinting, Std-FP (420 mg/L)

Weigh 4.2 mg of geniposidic acid CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 10 mL of methanol. Reflux the mixture for 30 min. Cool down to room temperature. Centrifuge at about 3000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	95 → 82	5 → 18	linear gradient
20 – 30	82	18	isocratic
30 – 60	82 → 52	18 → 48	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of acteoside Std-FP and geniposidic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and geniposidic acid should not be more than 5.0%; the RSD of the retention times of acteoside and geniposidic acid peaks should not be more than 2.0%; the column efficiencies determined from acteoside and geniposidic acid peaks should not be less than 20000 and 10000 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. 5).

Procedure

Separately inject acteoside Std-FP, geniposidic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of acteoside and geniposidic acid peaks in the chromatograms of acteoside Std-FP, geniposidic acid Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify acteoside and geniposidic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of acteoside Std-FP and geniposidic acid Std-FP. The retention times of acteoside and geniposidic acid 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 four characteristic peaks of Plantaginis Semen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Plantaginis Semen extract

Peak No.	RRT	Acceptable Range
1 (geniposidic acid)	0.32	± 0.03
2 (marker, acteoside)	1.00	-
3	1.17	± 0.03
4	1.23	± 0.05

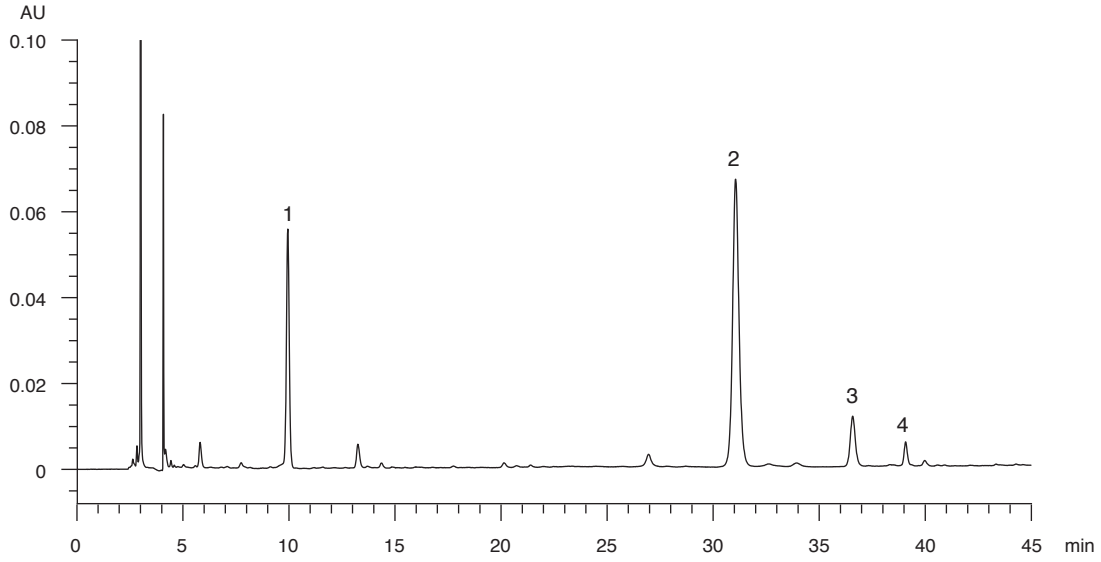


Figure 5 A reference fingerprint chromatogram of Plantaginis Semen extract

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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
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

Mixed acteoside and geniposidic acid standard stock solution, Std-Stock (500 mg/L each)

Weigh accurately 1.0 mg of acteoside CRS and 1.0 mg of geniposidic acid CRS, and dissolve in 2 mL of methanol (60%).

Mixed acteoside and geniposidic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed acteoside and geniposidic acid Std-Stock, dilute with methanol (60%) to produce a series of solutions of 10, 20, 40, 60, 100 mg/L for both acteoside and geniposidic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 30 mL of methanol (60%). Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue for three times each with 5 mL of methanol (60%). Combine the solutions and make up to the mark with methanol (60%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (330 nm for acteoside and 250 nm for geniposidic acid) 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)	0.5% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 50	95 → 52	5 → 48	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed acteoside and geniposidic acid Std-AS (40 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of acteoside and geniposidic acid should not be more than 5.0%; the RSD of the retention times of acteoside and geniposidic acid peaks should not be more than 2.0%; the column efficiencies determined from acteoside and geniposidic acid peaks should not be less than 100000 and 25000 theoretical plates respectively.

The *R* value between acteoside peak and the closest peak; and the *R* value between geniposidic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

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

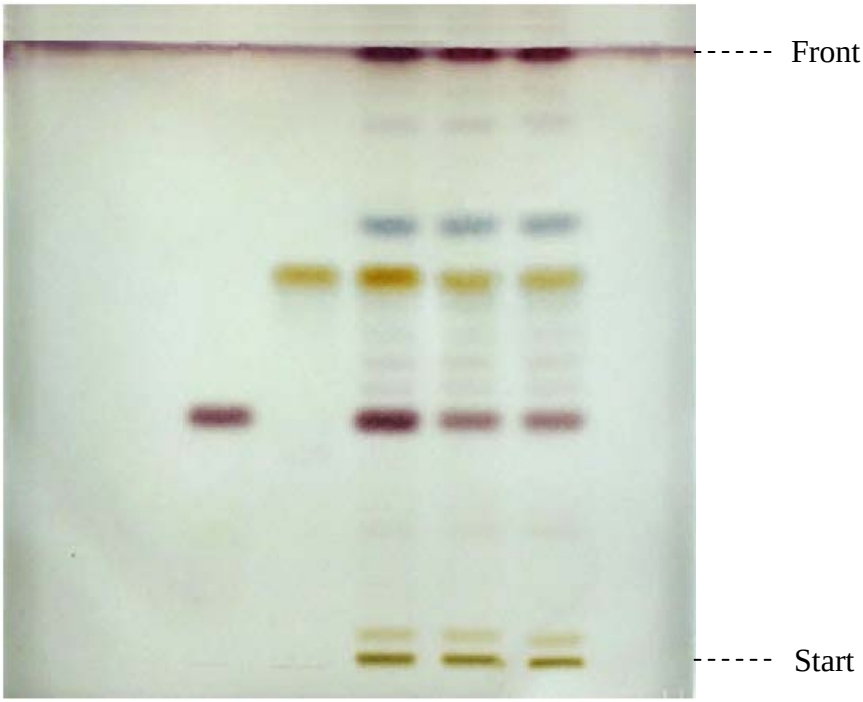
Procedure

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

Limits

The sample contains not less than 0.63% of acteoside ($C_{29}H_{36}O_{15}$) and geniposidic acid ($C_{16}H_{22}O_{10}$) respectively, calculated with reference to the dried substance.

Plantaginis Semen (車前子)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (Geniposidic acid)	Geniposidic acid positive
3	Standard (Acteoside)	Acteoside positive
4	Spiked sample (Sample plus geniposidic acid and acteoside)	Geniposidic acid and acteoside positive
5	Sample (Plantaginis Semen)	Geniposidic acid and acteoside positive
6	Sample duplicate (Plantaginis Semen)	Geniposidic acid and acteoside positive

Figure 1 TLC results of Plantaginis Semen extract observed under visible light after staining