Nelumbinis Stamen

Figure 1 A photograph of Nelumbinis Stamen

A. Nelumbinis Stamen B. Magnified image of stamens

1. NAMES

Official Name: Nelumbinis Stamen

Chinese Name: 蓮鬚

Chinese Phonetic Name: Lianxu

2. SOURCE

Nelumbinis Stamen is the dried stamen of *Nelumbo nucifera* Gaertn. (Nymphaeaceae). The stamen is collected at flowering period on sunny days in summer, dried under the sun under paper cover, or in shaded area to obtain Nelumbinis Stamen.

3. DESCRIPTION

Linear. Anthers twisted, longitudinally split, 1.2-1.8 cm long, about 1 mm in diameter, pale yellow, yellowish-brown to reddish-brown. Filaments slender, slightly curved, 1.0-2.2 cm long, pale purple, yellowish-purple to reddish-purple. Texture lax. Odour slightly aromatic; taste astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells rectangular, polygonal or irregular in shape, covered with cuticle. Connective composed of 1 vascular bundle and parenchymatous cells. 1 locule divided into 2 pollen sacs, endothecium slightly thickened and beaded; each pollen sac is lined with residual tapetum; some pollen grains scattered in pollen sac, subspherical or oblong (Fig. 2).

Powder

Colour yellowish-brown to reddish-brown. Epidermal cells of connective subrounded to rounded in surface view, anticlinal walls sinuous; outer walls papillated in lateral view. Epidermal cells of anther rectangular, polygonal or irregular in shape in surface view; outer walls wavy in lateral view and with distinct cuticle striations. Endothecium cells strip-shaped, walls slightly thickened and beaded, lumens sometimes contain yellowish-brown or reddish-brown contents. Pollen grains subspherical to oblong, 45-81 μm in diameter, composed of two outer walls, with tricolpate and granular reticulate striations on the surface. Parenchymatous cells of filaments and connective vary in shape, walls thin and sinuous, containing yellow, yellowish-brown or reddish-brown contents. Clusters of calcium oxalate sometimes visible and usually aggregated in rosette-shape; polychromatic under the polarized microscope. Vessels mainly spiral, 4-19 μm in diameter, double-spiral and annular vessels sometimes visible (Fig. 3).

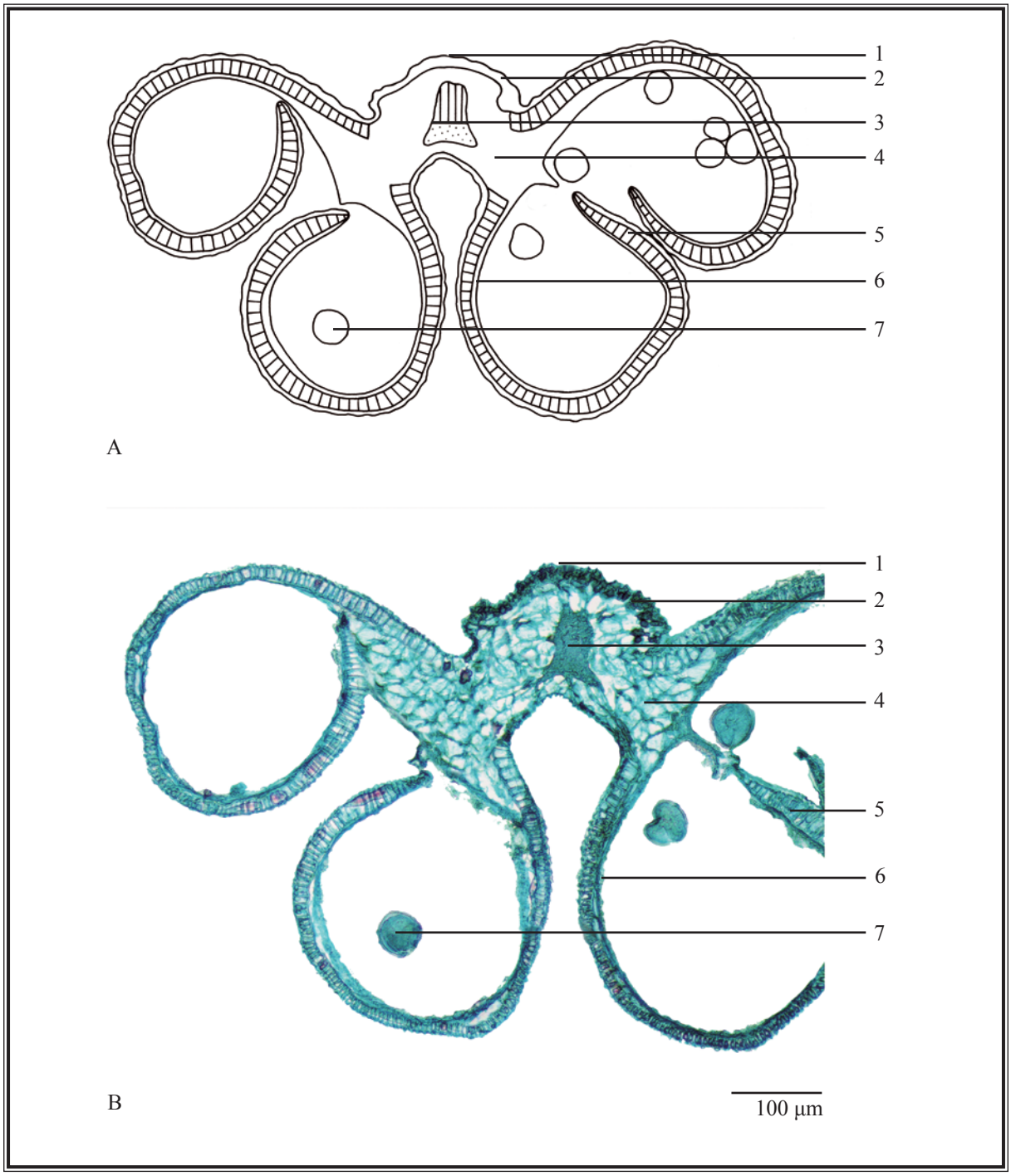

Figure 2 Microscopic features of transverse section of anther of Nelumbinis Stamen

A. Sketch B. Section illustration

1. Cuticle 2. Epidermis 3. Vascular bundle 4. Connective parenchymatous cells
5. Endothecium 6. Residual tapetum 7. Pollen grains

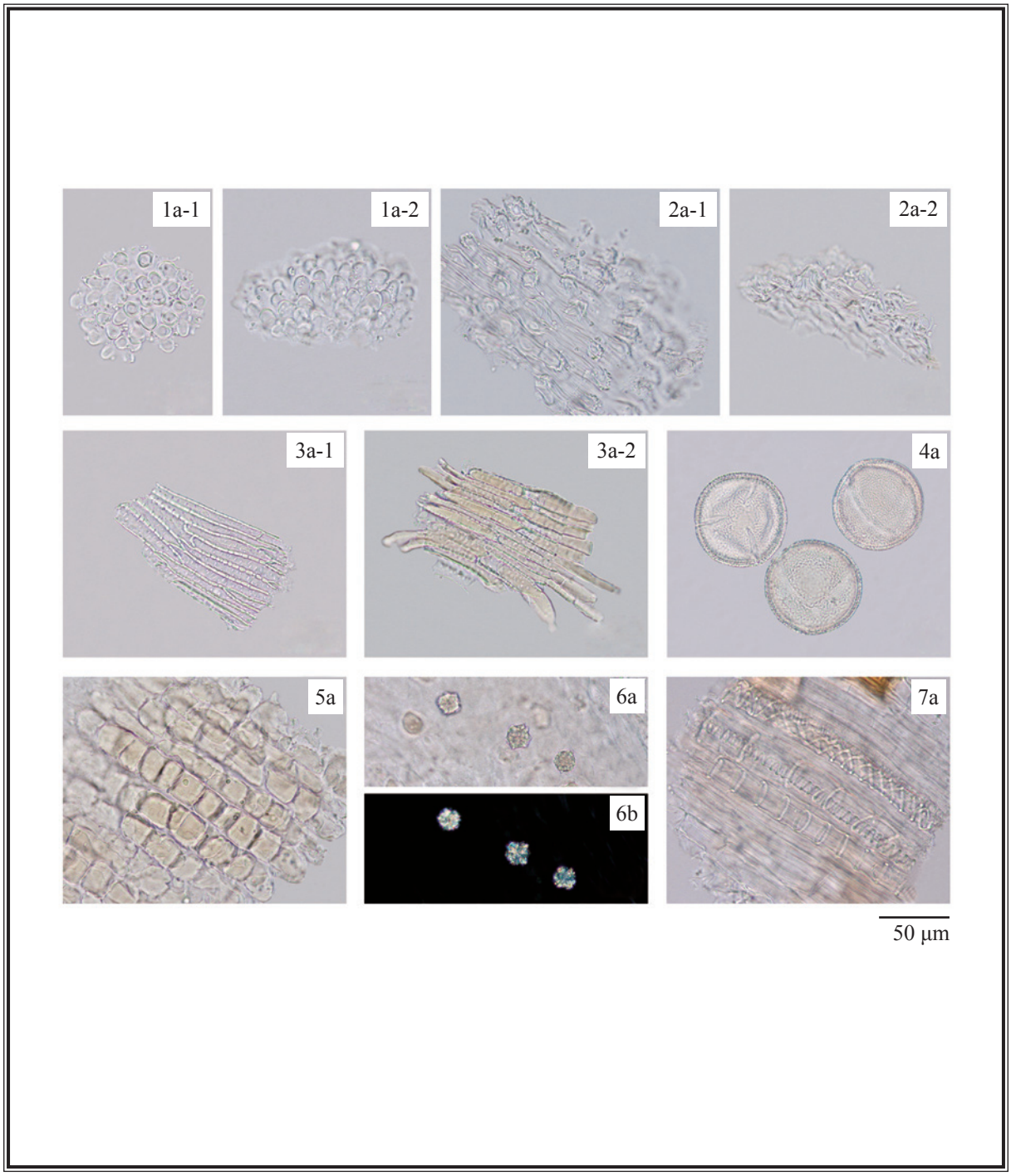

Figure 3 Microscopic features of powder of Nelumbinis Stamen

1. Epidermal cells of connective (1-1 in surface view, 1-2 in lateral view)
2. Epidermal cells of anther (2-1 in surface view, 2-2 in lateral view)
3. Endothecium cells (3-1 without contents, 3-2 with yellowish-brown contents)
4. Pollen grains 5. Parenchymatous cells of filaments and connective
6. Clusters of calcium oxalate 7. Vessels

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

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

Standard solutions

Astragalin standard solution

Weigh 0.5 mg of astragalin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Kaempferol standard solution

Weigh 0.5 mg of kaempferol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane, formic acid and water (6.5:2.5:1:0.5, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (400 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 15 min. 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 astragalin standard solution (1 μL), kaempferol 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. Spray the plate evenly with the spray reagent and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

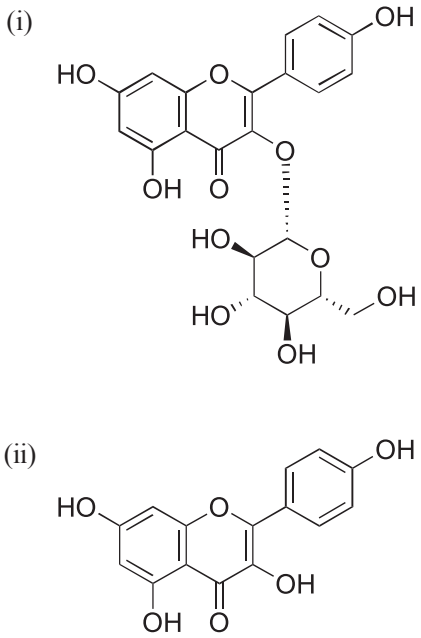

Figure 4 Chemical structures of (i) astragalin and (ii) kaempferol

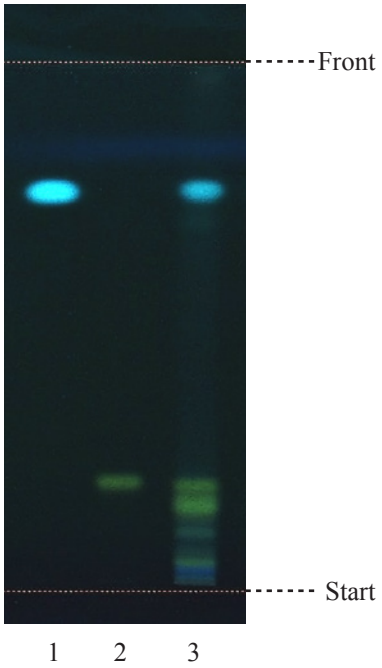

Figure 5 A reference HPTLC chromatogram of Nelumbinis Stamen extract observed under UV light (366 nm) after staining

1. Kaempferol standard solution 2. Astragalin 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 astragalin and kaempferol (Fig. 5).

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

Standard solutions

Astragalin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of astragalin CRS and dissolve in 10 mL of ethanol (75%).

Kaempferol standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.05 mg of kaempferol CRS and dissolve in 10 mL of ethanol (75%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (75%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 15 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (75%). Combine the solutions and make up to the mark with ethanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (3.5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 15	20	80	isocratic
15 – 50	20 → 63	80 → 37	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of astragalin Std-FP and kaempferol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of astragalin and kaempferol should not be more than 5.0%; the RSD of the retention times of astragalin and kaempferol peaks should not be more than 2.0%; the column efficiencies determined from astragalin and kaempferol peaks should not be less than 50000 and 100000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject astragalin Std-FP, kaempferol Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of astragalin and kaempferol peaks in the chromatograms of astragalin Std-FP, kaempferol Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify astragalin and kaempferol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of astragalin Std-FP and kaempferol Std-FP. The retention times of astragalin and kaempferol 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 five characteristic peaks of Nelumbinis Stamen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Nelumbinis Stamen extract

Peak No.	RRT	Acceptable Range
1	0.73	± 0.03
2 (marker, astragalin)	1.00	-
3	1.05	± 0.03
4	1.57	± 0.03
5 (kaempferol)	1.84	± 0.03

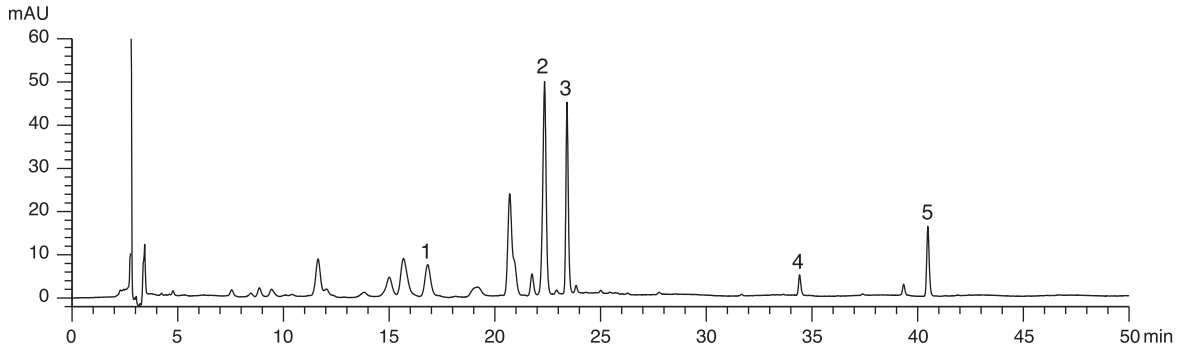

Figure 6 A reference fingerprint chromatogram of Nelumbinis Stamen extract

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. 6).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 18.0%.
Ethanol-soluble extractives (hot extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed astragalin and kaempferol standard stock solution, Std-Stock (320 mg/L for astragalin and 360 mg/L for kaempferol)

Weigh accurately 3.2 mg of astragalin CRS and 3.6 mg of kaempferol CRS, and dissolve in 10 mL of ethanol (75%).

Mixed astragalin and kaempferol standard solution for assay, Std-AS

Measure accurately the volume of the mixed astragalin and kaempferol Std-Stock, dilute with ethanol (75%) to produce a series of solutions of 5, 10, 20, 40, 80 mg/L for astragalin and 0.5, 1, 1.5, 3, 6 mg/L for kaempferol.

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 (75%). Sonicate (200 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 15 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (75%). Combine the solutions and make up to the mark with ethanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (360 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (3.5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 15	20	80	isocratic
15 – 50	20 → 63	80 → 37	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed astragalin and kaempferol Std-AS (20 mg/L for astragalin and 1.5 mg/L for kaempferol). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of astragalin and kaempferol should not be more than 5.0%; the RSD of the retention times of astragalin and kaempferol peaks should not be more than 2.0%; the column efficiencies determined from astragalin and kaempferol peaks should not be less than 50000 and 100000 theoretical plates respectively.

The *R* value between astragalin peak and the closest peak; and the *R* value between kaempferol 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 astragalin and kaempferol Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of astragalin and kaempferol against the corresponding concentrations of the mixed astragalin and kaempferol 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 astragalin and kaempferol peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed astragalin and kaempferol Std-AS. The retention times of astragalin and kaempferol 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 astragalin and kaempferol in the test solution, and calculate the percentage contents of astragalin and kaempferol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.12% of the total content of astragalin ($C_{21}H_{20}O_{11}$) and kaempferol ($C_{15}H_{10}O_6$), calculated with reference to the dried substance.