

Daturae Flos



Figure 1 A photograph of Daturae Flos

- A. Daturae Flos B. Magnified image of dried flower
C. Magnified image of inner view of dissected flower

1. NAMES

Official Name: Daturae Flos

Chinese Name: 洋金花

Chinese Phonetic Name: Yangjinhua

2. SOURCE

Daturae Flos is the dried flower of *Datura metel* L. (Solanaceae). The flower is collected at the beginning of flowering period in March to November, then dried under the sun or at an ambient temperature to obtain Daturae Flos.

3. DESCRIPTION

Usually crumpled and strip-shaped, 8-16 cm long. Calyx tubular, 2/5 in length of corolla, greyish-green to greyish-yellow, apex 5-lobed, with 5 longitudinal veins at the base, surface slightly pubescent; corolla trumpet-shaped, pale yellow or yellowish-brown, apex slightly 5-lobed, lobes short acuminate, with 3 distinct longitudinal veins below the acumen, slightly sunken between two lobes; stamens 5, filaments adnate to the corolla tube, 3/4 in length of corolla; pistil 1, stigma stick-shaped. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Powder

Colour pale yellow. Pollen grains spherical to subspherical, 42-65 μm in diameter, with stripe-shaped sculptures on the exine, 3 germinal pores occasionally visible. Clusters of calcium oxalate scattered singly in parenchymatous cells, 7-40 μm in diameter; polychromatic under the polarized microscope. Spiral vessels 14-35 μm in diameter. Non-glandular hairs commonly visible on the surface of corolla and calyx, composed of 1-5 cells, one cell may be collapsed, forming a dumbbell shape. Glandular hairs not commonly seen (Fig. 2).

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

Lonicerae Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

杠板歸

北豆根
Menispermii Rhizoma

山銀花

Plumbaginis Zeylanicae Radix

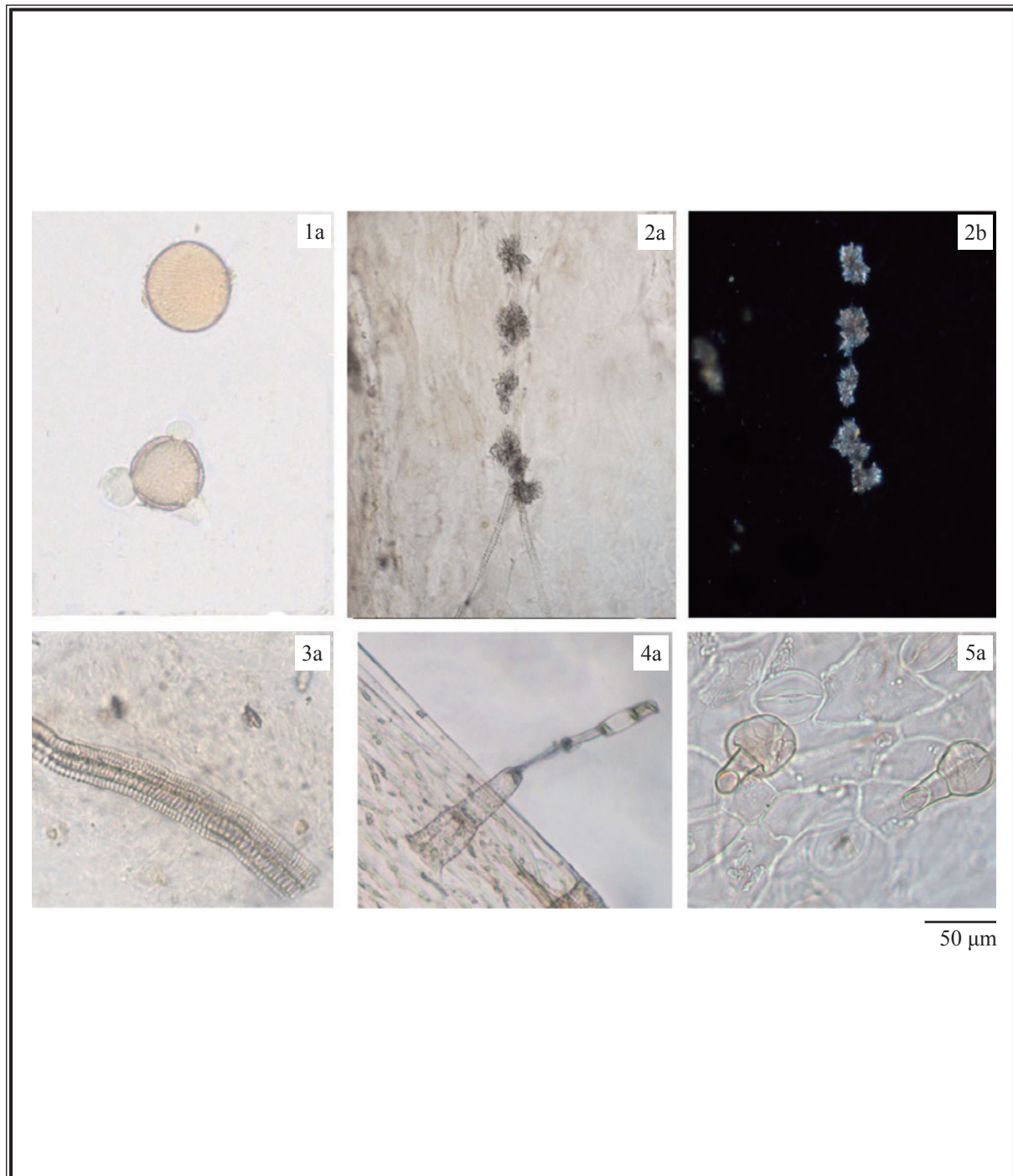
Daturae Flos

Figure 2 Microscopic features of powder of *Daturae Flos*

1. Pollen grains
2. Clusters of calcium oxalate
3. Spiral vessels
4. Non-glandular hair
5. Glandular hairs

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

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

Standard solutions

L-Hyoscyamine sulphate dihydrate standard solution

Weigh 1.0 mg of L-hyoscyamine sulphate dihydrate CRS (Fig. 3) and dissolve in 1 mL of methanol. Keep at about 4°C.

Scopolamine hydrobromide standard solution

Weigh 1.0 mg of scopolamine hydrobromide CRS (Fig. 3) and dissolve in 1 mL of methanol. Keep at about 4°C.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), methanol and ethyl acetate (0.5:1:8.5, v/v).

Spray reagent

Solution A

Weigh 0.85 g of bismuth oxynitrate and dissolve in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

Solution B

Weigh 4 g of potassium iodide and dissolve in 10 mL of water.

Spray reagent

Transfer 5 mL of Solution A, 5 mL of Solution B and 20 mL of glacial acetic acid into a 100-mL volumetric flask and make up to the mark with water. 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 1 mL of ammonium hydroxide solution (25%, v/v) and 25 mL of a mixture of ethyl acetate and methanol (4:1, v/v). Sonicate (500 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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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 L-hyoscyamine sulphate dihydrate standard solution, scopolamine hydrobromide standard solution and the test solution (5 μ L each) 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 7 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 visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

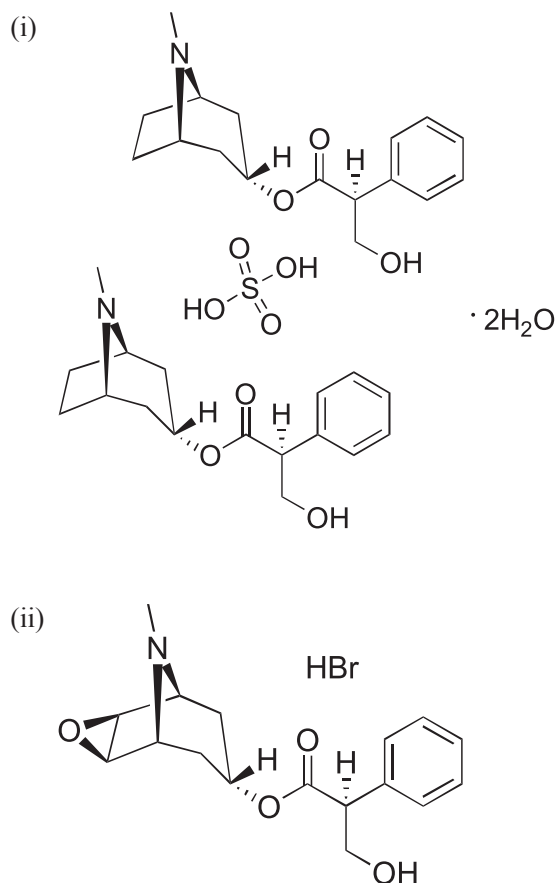


Figure 3 Chemical structures of (i) L-hyoscyamine sulphate dihydrate and (ii) scopolamine hydrobromide

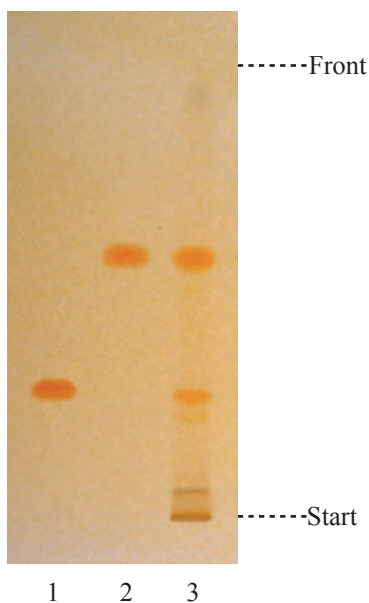


Figure 4 A reference HPTLC chromatogram of *Daturae Flos* extract observed under visible light after staining

1. L-Hyoscyamine sulphate dihydrate standard solution
2. Scopolamine hydrobromide 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 L-hyoscyamine and scopolamine (Fig. 4).

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

Reagents

0.005 M Sodium 1-heptanesulphonate solution

Weigh 0.5 g of sodium 1-heptanesulphonate and dissolve in 500 mL of water.

0.1 M Potassium dihydrogen phosphate solution

Weigh 6.8 g of potassium dihydrogen phosphate and dissolve in 500 mL of water.

Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5)

Transfer 500 mL of 0.005 M sodium 1-heptanesulphonate solution and 500 mL of 0.1 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 5 with 0.04 M potassium hydroxide solution.

Standard solutions

L-Hyoscyamine sulphate dihydrate standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of L-hyoscyamine sulphate dihydrate CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

Scopolamine hydrobromide standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of scopolamine hydrobromide CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 2 mL of ammonium hydroxide solution (25%, v/v) and 20 mL of a mixture of ethyl acetate and methanol (4:1, v/v). Sonicate (500 W) the mixture for 30 min. Centrifuge at about $4000 \times g$ for 10 min. Transfer the supernatant to a 150-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator in a water bath at about 40°C. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (216 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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)	Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5) (% , v/v)	Acetonitrile : Methanol (10:1, v/v) (% , v/v)	Elution
0 – 10	90 → 80	10 → 20	linear gradient
10 – 40	80 → 70	20 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μ L of L-hyoscyamine sulphate dihydrate Std-FP and scopolamine hydrobromide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of L-hyoscyamine and scopolamine should not be more than 5.0%; the RSD of the retention times of L-hyoscyamine and scopolamine peaks should not be more than 2.0%; the column efficiencies determined from L-hyoscyamine and scopolamine peaks should not be less than 60000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject L-hyoscyamine sulphate dihydrate Std-FP, scopolamine hydrobromide Std-FP and the test solution (5 μ L each) into the HPLC system and record the chromatograms. Measure the retention times of L-hyoscyamine and scopolamine peaks in the chromatograms of L-hyoscyamine sulphate dihydrate Std-FP, scopolamine hydrobromide Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify L-hyoscyamine and scopolamine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of L-hyoscyamine sulphate dihydrate Std-FP and scopolamine hydrobromide Std-FP. The retention times of L-hyoscyamine and scopolamine 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 Daturae Flos extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Daturae Flos extract

Peak No.	RRT	Acceptable Range
1	0.48	± 0.03
2 (marker, scopolamine)	1.00	-
3 (L-hyoscyamine)	1.21	± 0.03
4	1.69	± 0.05

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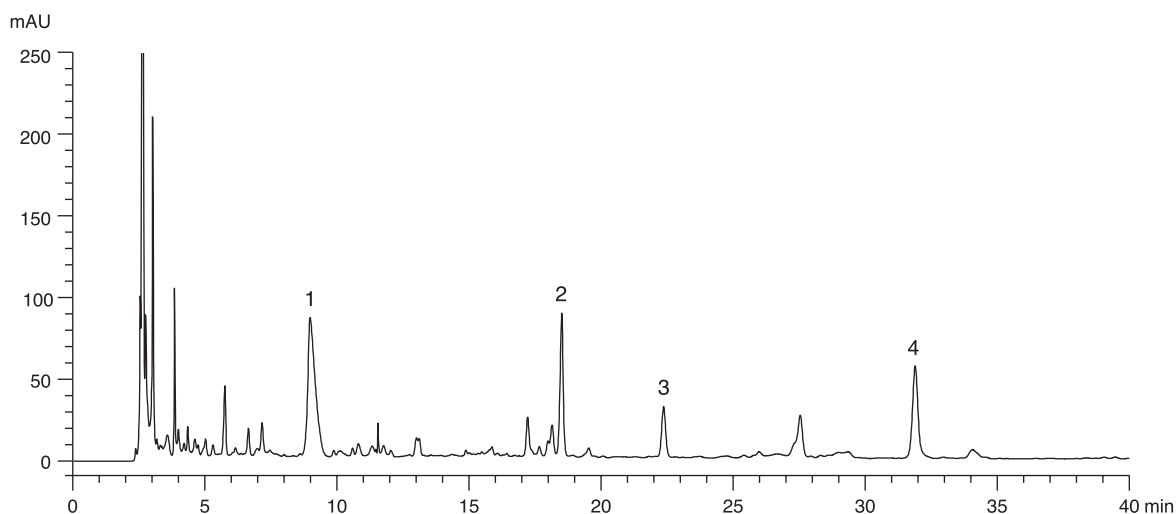
Daturae Flos

Figure 5 A reference fingerprint chromatogram of *Daturae Flos* 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 (*Appendix VII*): meet the requirements.

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

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

5.6 Ash (*Appendix IX*)

Total ash: not more than 15.0%.

Acid-insoluble ash: not more than 2.0%.

5.7 Water Content (*Appendix X*)

Oven dried method: not more than 9.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 26.0%.

Ethanol-soluble extractives (hot extraction method): not less than 15.0%.

7. ASSAY

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

Reagents

0.005 M Sodium 1-heptanesulphonate solution

Weigh 0.5 g of sodium 1-heptanesulphonate and dissolve in 500 mL of water.

0.1 M Potassium dihydrogen phosphate solution

Weigh 6.8 g of potassium dihydrogen phosphate and dissolve in 500 mL of water.

Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5)

Transfer 500 mL of 0.005 M sodium 1-heptanesulphonate solution and 500 mL of 0.1 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 5 with 0.04 M potassium hydroxide solution.

Standard solution

Mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide standard stock solution, Std-Stock (1200 mg/L for L-hyoscyamine sulphate dihydrate and 2120 mg/L for scopolamine hydrobromide)

Weigh accurately 12.0 mg of L-hyoscyamine sulphate dihydrate CRS and 21.2 mg of scopolamine hydrobromide CRS, and dissolve in 10 mL of methanol. Keep at about 4°C.

Mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide standard solution for assay, Std-AS

Measure accurately the volume of the mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-Stock, dilute with methanol to produce a series of solutions of 30, 60, 120, 300, 600 mg/L for L-hyoscyamine sulphate dihydrate and 53, 106, 212, 530, 1060 mg/L for scopolamine hydrobromide. Keep at about 4°C.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 2 mL of ammonium hydroxide solution (25%, v/v) and 20 mL of a mixture of ethyl acetate and methanol (4:1, v/v). Sonicate (500 W) the mixture for 30 min. Centrifuge at about 4000 × g for 10 min. Transfer

the supernatant to a 150-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator in a water bath at about 40°C. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (216 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m 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)	Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5) (% v/v)	Acetonitrile : Methanol (10:1, v/v) (% v/v)	Elution
0 – 10	90 \rightarrow 80	10 \rightarrow 20	linear gradient
10 – 40	80 \rightarrow 70	20 \rightarrow 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μ L of the mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-AS (120 mg/L for L-hyoscyamine sulphate dihydrate and 212 mg/L for scopolamine hydrobromide). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of L-hyoscyamine and scopolamine should not be more than 5.0%; the RSD of the retention times of L-hyoscyamine and scopolamine peaks should not be more than 2.0%; the column efficiencies determined from L-hyoscyamine and scopolamine peaks should not be less than 60000 theoretical plates.

The *R* value between L-hyoscyamine peak and the closest peak; and the *R* value between scopolamine 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 L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-AS (5 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of L-hyoscyamine and scopolamine against the corresponding concentrations of the mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify L-hyoscyamine and scopolamine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-AS. The retention times of L-hyoscyamine and scopolamine 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 L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide in the test solution, and calculate the percentage contents of L-hyoscyamine (the percentage content of L-hyoscyamine sulphate dihydrate × 0.41, where 0.41 is the molar mass ratio of L-hyoscyamine and L-hyoscyamine sulphate dihydrate) and scopolamine (the percentage content of scopolamine hydrobromide × 0.79, where 0.79 is the molar mass ratio of scopolamine and scopolamine hydrobromide) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.036% of L-hyoscyamine (C₁₇H₂₃NO₃) and not less than 0.19% of scopolamine (C₁₇H₂₁NO₄), calculated with reference to the dried substance.

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.