Hyoscyami Semen (unprocessed)

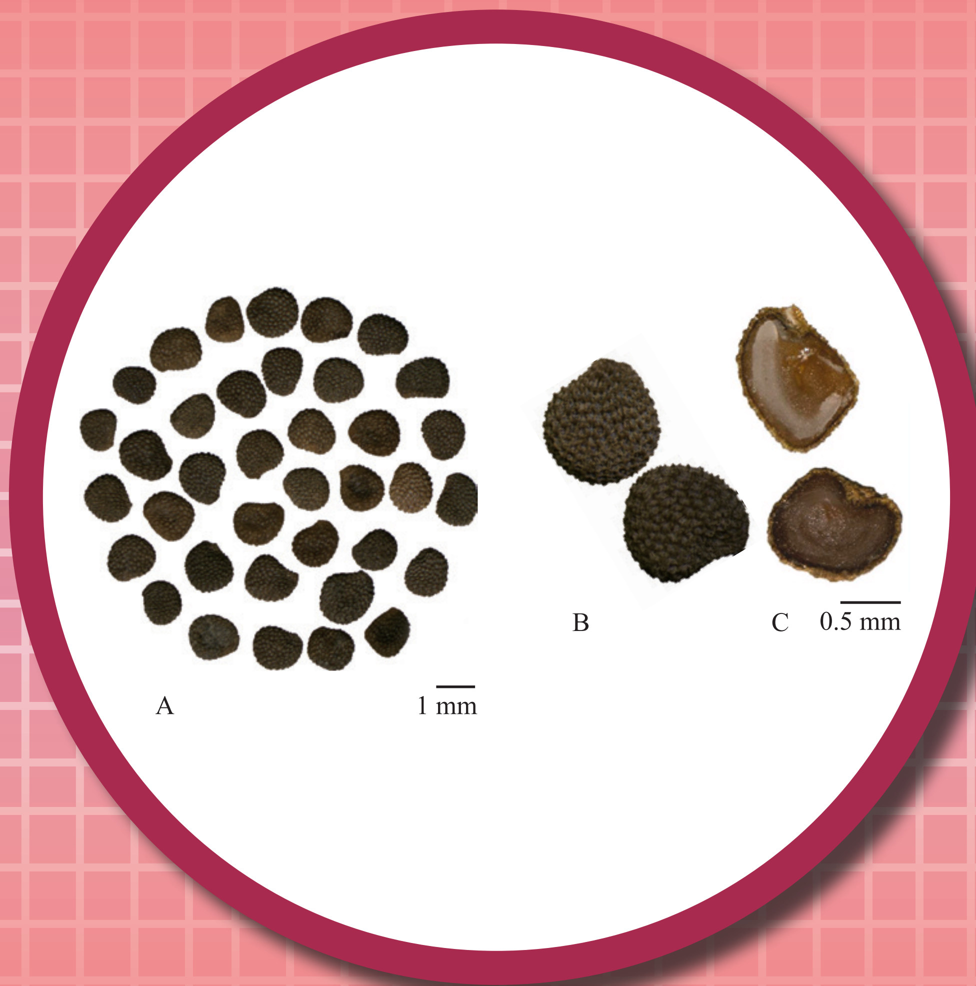

Figure 1 A photograph of Hyoscyami Semen (unprocessed)

A. Hyoscyami Semen (unprocessed)

B. Magnified image of seeds

C. Magnified image of longitudinal section of seeds

1. NAMES

Official Name: Hyoscyami Semen (unprocessed)

Chinese Name: 天仙子（生）

Chinese Phonetic Name: Tianxianzi (Sheng)

2. SOURCE

Hyoscyami Semen (unprocessed) is the unprocessed dried ripe seed of *Hyoscyamus niger* L. (Solanaceae). The fruit is collected in summer and autumn when the pericarp turns yellow, exposed to the sun, afterwards the seeds tapped out, pericarp and stalk removed, then dried under the sun to obtain Hyoscyami Semen (unprocessed).

3. DESCRIPTION

Flattened-subreniform to flattened-ovoid, about 1 mm in diameter. Externally brownish-yellow or greyish-yellow, with fine and dense reticulate striations, and a pitted hilum located at the slightly acute end. Cut surface greyish-white, oily, containing endosperm, embryo curved. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Exotesta cells with irregularly undulant protuberances, apex of the protuberances tapering or blunt, cell walls with transparent striations. Endotesta consists of 1 layer of cells, cells mostly tangentially elongated, with thin and slightly wizened walls, containing brown contents. Endosperm cells with slightly thickened walls, containing aleurone grains. Cotyledon cells with thin walls, containing oil droplets (Fig. 2).

Powder

Exotesta cells scattered or in groups, yellow or greyish-yellowish brown, polygonal or elongated polygonal in surface view, 75-311 µm long, 25-123 µm in diameter, anticlinal walls thickened, undulant, with distinct striations, containing yellowish-brown contents; bright yellowish-white under the polarized microscope. Endosperm cells polygonal or subrounded in surface view, cell walls slightly thickened, containing aleurone grains and oil droplets. Cotyledon cells colourless, subpolygonal, with thin walls, containing oil droplets (Fig. 3).

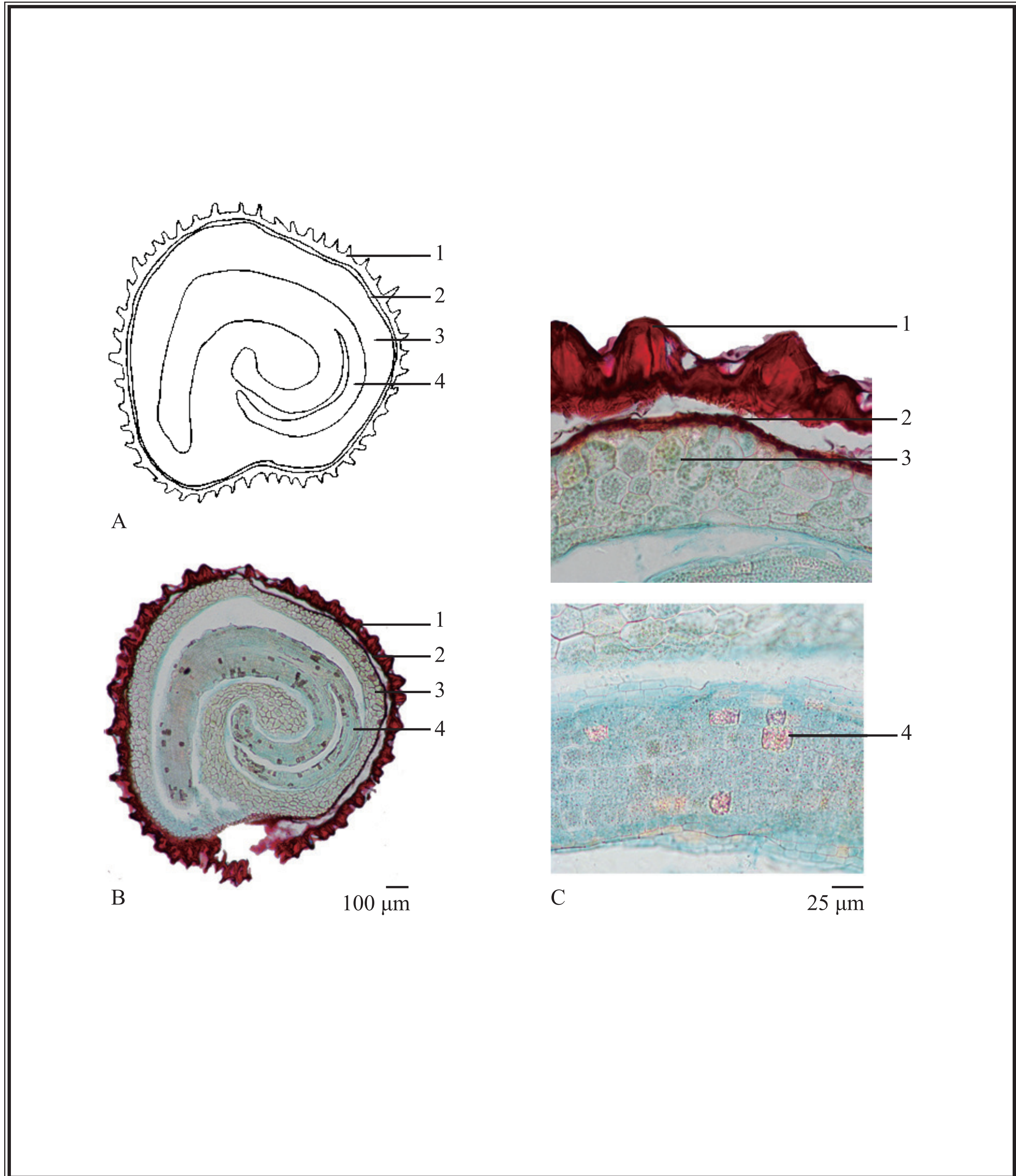

Figure 2 Microscopic features of transverse section of Hyoscyami Semen (unprocessed)

A. Sketch B. Section illustration C. Section magnified

1. Exotesta 2. Endotesta 3. Endosperm 4. Cotyledon

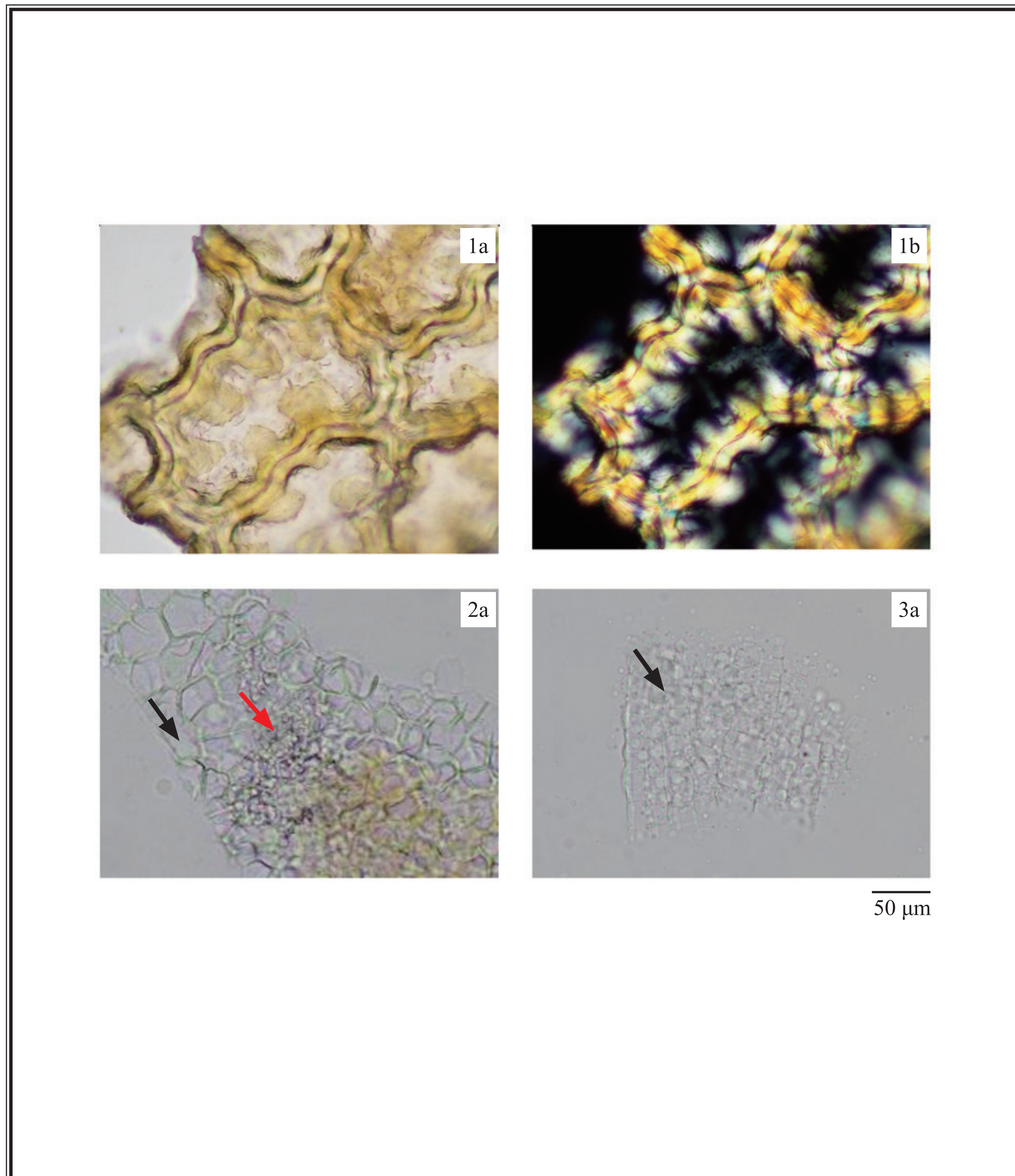

Figure 3 Microscopic features of powder of Hyoscyami Semen (unprocessed)

1. Exotesta cells 2. Endosperm cells (oil droplets →, aleurone grains →)
3. Cotyledon cells (oil droplets →)

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. 4) and dissolve in 1 mL of methanol. Keep at about 4ºC.

Scopolamine hydrobromide standard solution

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

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 1

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.

Spray reagent 2

Weigh 5 g of sodium nitrite and dissolve in 50 mL of ethanol (60%).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 15 mL of petroleum ether (30-60ºC). Sonicate (160 W) the mixture for 20 min. Filter and discard the filtrate. Repeat the extraction for one more time. Dissolve the residue in 2 mL ammonium hydroxide solution (25%, v/v) and 30 mL of ethanol. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Wash the residue for three times each with 10 mL of ethanol. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 (3 μL), scopolamine hydrobromide standard solution (3 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 heat at about 105ºC (about 10 min). Spray the plate evenly with the spray reagent 1 and the spray reagent 2, then dry in air. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

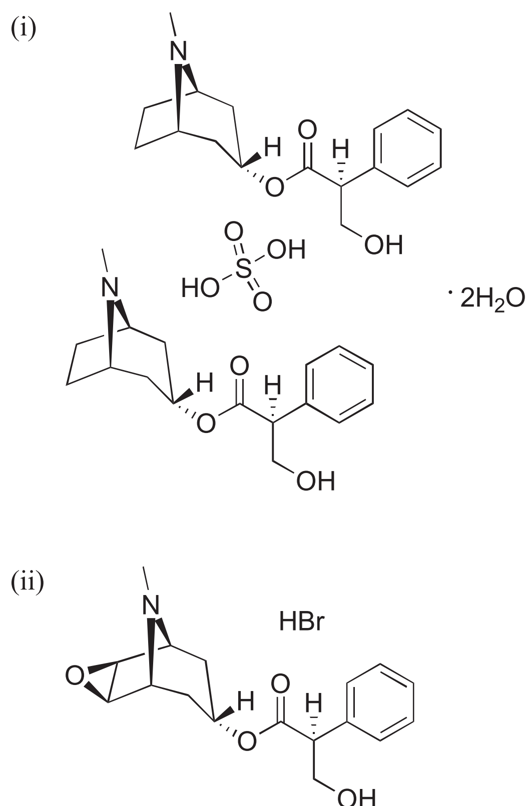

Figure 4 Chemical structures of (i) L-hyoscyamine sulphate dihydrate and (ii) scopolamine hydrobromide

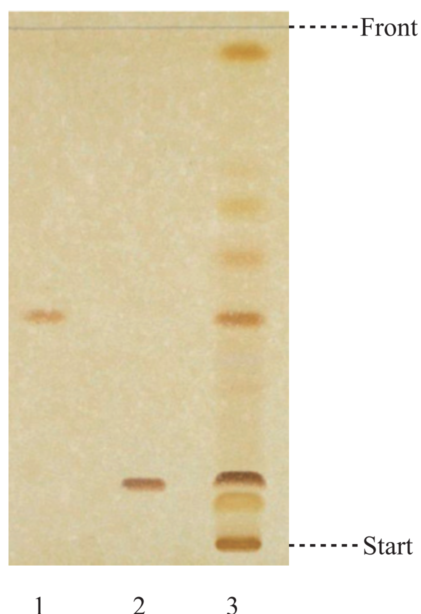

Figure 5 A reference HPTLC chromatogram of Hyoscyami Semen (unprocessed) extract observed under visible light after staining

1. Scopolamine hydrobromide standard solution
2. L-Hyoscyamine sulphate dihydrate 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. 5).

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.

0.01 M Potassium dihydrogen phosphate solution

Weigh 0.68 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 0.01 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 5 with 1.6 M potassium hydroxide solution.

Standard solutions

L-Hyoscyamine sulphate dihydrate standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of L-hyoscyamine sulphate dihydrate CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

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

Weigh 0.2 mg of scopolamine hydrobromide CRS and dissolve in 10 mL of methanol. Keep at about 4ºC.

Test solution

Weigh 1.0 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 200 mL of petroleum ether (30-60ºC) to a 250-mL round-bottomed flask. Perform the soxhlet extraction for 2 h. Discard the petroleum ether (30-60ºC) and dry the residue in air. Transfer the dried residue to a 250-mL round-bottomed flask and add 5 mL of ammonium hydroxide solution (25%, v/v) and 75 mL of ethanol. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue with ethanol. Combine the solution and make up to the mark with ethanol. Pipette 20 mL of the solution to a 50-mL conical flask. Adjust the pH to 5 with hydrochloric acid solution (10%, v/v). Pre-condition a solid-phase extraction column (strong cation exchange, 6 mL, 500 mg) with 3 mL of methanol, 3 mL of a mixture of ammonium hydroxide solution (25%, v/v) and ethanol (1:15, v/v), then followed by 3 mL of ethanol and 5 mL of ethanol [adjust the pH to 4 with hydrochloric acid solution (10%, v/v)]. Allow to stand for 10 min. Load the sample solution to the pre-conditioned SPE column. Add 3 mL of ethanol to the column and discard the eluant. Add 5 mL of a mixture of ammonium hydroxide solution (25%, v/v) and ethanol (1:15, v/v) to the column. Collect the eluant in a 100-mL round-bottomed flask. Evaporate the solvent to near dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC 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)	Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5) (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	90 → 82	10 → 18	linear gradient
10 – 30	82 → 77	18 → 23	linear gradient
30 – 35	77	23	isocratic
35 – 40	77 → 73	23 → 27	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 µL 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 20000 theoretical plates.

The *R* value between peak 3 and the closest peak; and 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. 6).

Procedure

Separately inject L-hyoscyamine sulphate dihydrate Std-FP, scopolamine hydrobromide Std-FP and the test solution (5 µL 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. 6) 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 Hyoscyami Semen (unprocessed) extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Hyoscyami Semen (unprocessed) extract

Peak No.	RRT	Acceptable Range
1	0.54	± 0.03
2	0.61	± 0.03
3 (marker, scopolamine)	1.00	-
4 (L-hyoscyamine)	1.27	± 0.03

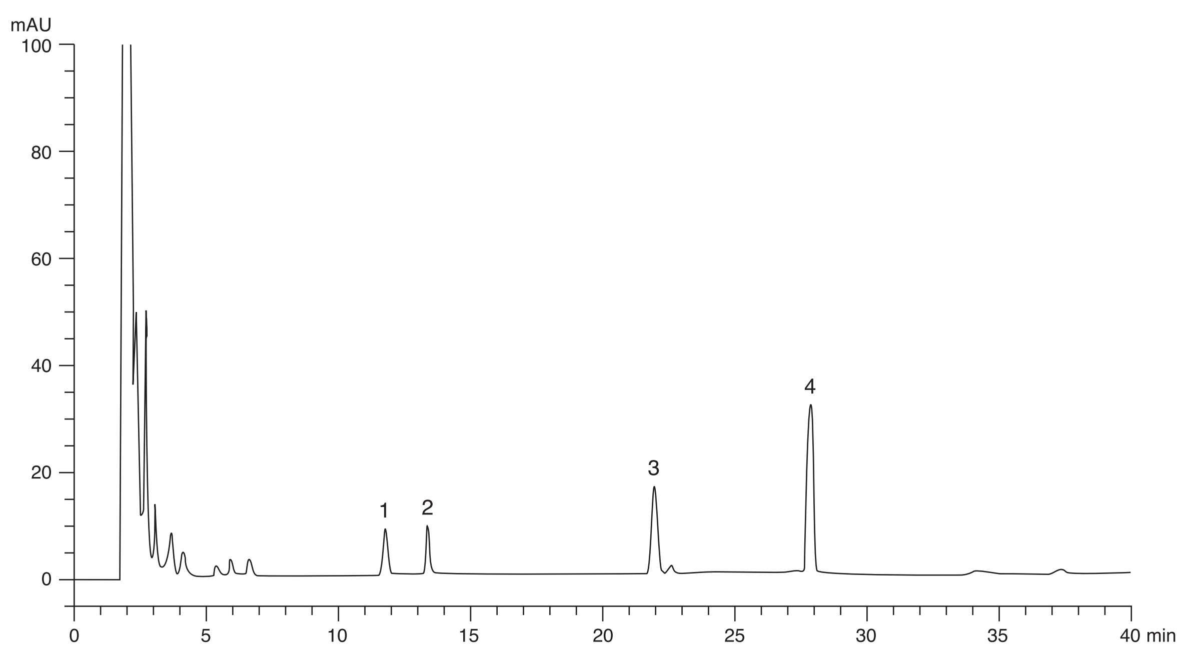

Figure 6 A reference fingerprint chromatogram of Hyoscyami Semen (unprocessed) extract

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. 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 3.0%.

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 2.5%.

5.7 **Water Content** *(Appendix X)*

Oven dried method: not more than 8.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

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.
0.01 M Potassium dihydrogen phosphate solution
Weigh 0.68 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 0.01 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 5 with 1.6 M potassium hydroxide solution.

Standard solution

Mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of L-hyoscyamine sulphate dihydrate CRS and 2.0 mg of scopolamine hydrobromide CRS, and dissolve in 10 mL of methanol. Keep at about 4ºC.

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 10, 60, 120, 160, 200 mg/L for both L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide. Keep at about 4ºC.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 200 mL of petroleum ether (30-60ºC) to a 250-mL round-bottomed flask. Perform the soxhlet extraction for 2 h. Discard the petroleum ether (30-60ºC) and dry the residue in air. Transfer the dried residue to a 250-mL round-bottomed flask and add 5 mL of ammonium hydroxide solution (25%, v/v) and 75 mL of ethanol. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue with ethanol. Combine the solution and make up to the mark with ethanol. Pipette 20 mL of the solution to a 50-mL conical flask. Adjust the pH to 5 with hydrochloric acid solution (10%, v/v). Pre-condition a solid-phase extraction column (strong cation exchange, 6 mL, 500 mg) with 3 mL of methanol, 3 mL of a mixture of ammonium hydroxide solution (25%, v/v) and ethanol (1:15, v/v), then followed by 3 mL of ethanol and 5 mL of ethanol [adjust the pH to 4 with hydrochloric acid solution (10%, v/v)]. Allow to stand for 10 min. Load the sample solution to the pre-conditioned SPE column. Add 3 mL of ethanol to the column and discard the eluant. Add 5 mL of a mixture of ammonium hydroxide solution (25%, v/v) and ethanol (1:15, v/v) to the column. Collect the eluant in a 100-mL round-bottomed flask. Evaporate the solvent to near dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC 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)	Sodium 1-heptanesulphonate - potassium dihydrogen phosphate buffer solution (pH 5) (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	90 → 82	10 → 18	linear gradient
10 – 30	82 → 77	18 → 23	linear gradient
30 – 35	77	23	isocratic
35 – 40	77 → 73	23 → 27	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of the mixed L-hyoscyamine sulphate dihydrate and scopolamine hydrobromide Std-AS (120 mg/L each). 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 20000 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 μL 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 0.018% of L-hyoscyamine ($C_{17}H_{23}NO_3$) and not less than 0.026% of scopolamine ($C_{17}H_{21}NO_4$), calculated with reference to the dried substance.

8. CAUTION

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