Strychni Semen (unprocessed)

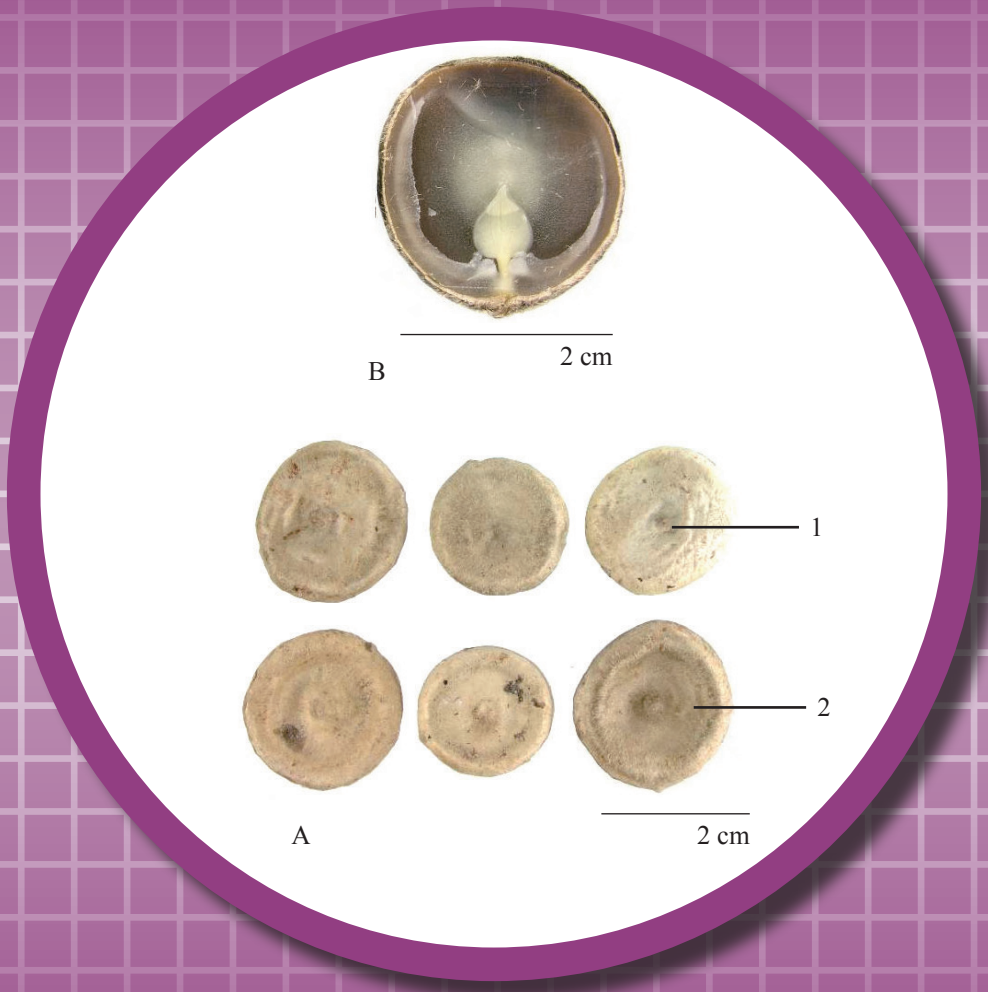


Figure 1 A photograph of Strychni Semen (unprocessed)

A. Strychni Semen (unprocessed) (1.Top view 2. Bottom view)
B. Magnified image of longitudinal section of ripe seed

1. NAMES

Official Name: Strychni Semen (unprocessed)

Chinese Name: 馬錢子 (生)

Chinese Phonetic Name: Maqianzi (Sheng)

2. SOURCE

Strychni Semen (unprocessed) is the unprocessed dried ripe seed of *Strychnos nux-vomica* L. (Loganiaceae). The fruit is collected in winter, and the seeds gathered, then dried under the sun to obtain Strychni Semen (unprocessed).

3. DESCRIPTION

Flattened and rounded, button-shaped, usually one side convex and the other slightly concave, 10-30 mm in diameter, 2-6 mm thick. Externally greyish-brown or greyish-green, densely covered with silver-grey pubescence, radiating from centre to the edges, silky-lustrous. Edges slightly prominent, relatively thick, with a protuberant micropyle. A prominent dotted hilum located in the centre of the bottom surface. Texture hard, pale yellowish-white endosperm visible in horizontal (longitudinal) section of the seed, corneous, cotyledons cordate, with 5-7 veins (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermal cells of testa differentiated into unicellular non-glandular hairs, which arise and extend obliquely at one side, 440-1112 μm long, 20-35 μm in diameter (measured in the middle of non-glandular hairs), enlarged at base, with extremely thick and lignified wall. Inner layer of testa consists of brown decadent parenchymatous cells. Endosperm cells polygonal, with thick wall, containing fatty oil and aleurone grains (Fig. 2).

Powder

Colour greyish-yellow. Non-glandular hairs unicellular, mostly broken, enlarged at base, walls extremely thick, lignified; bright white to polychromatic under the polarized microscope. Endosperm cells polygonal, walls thick, containing fatty oil and aleurone grains (Fig. 3).

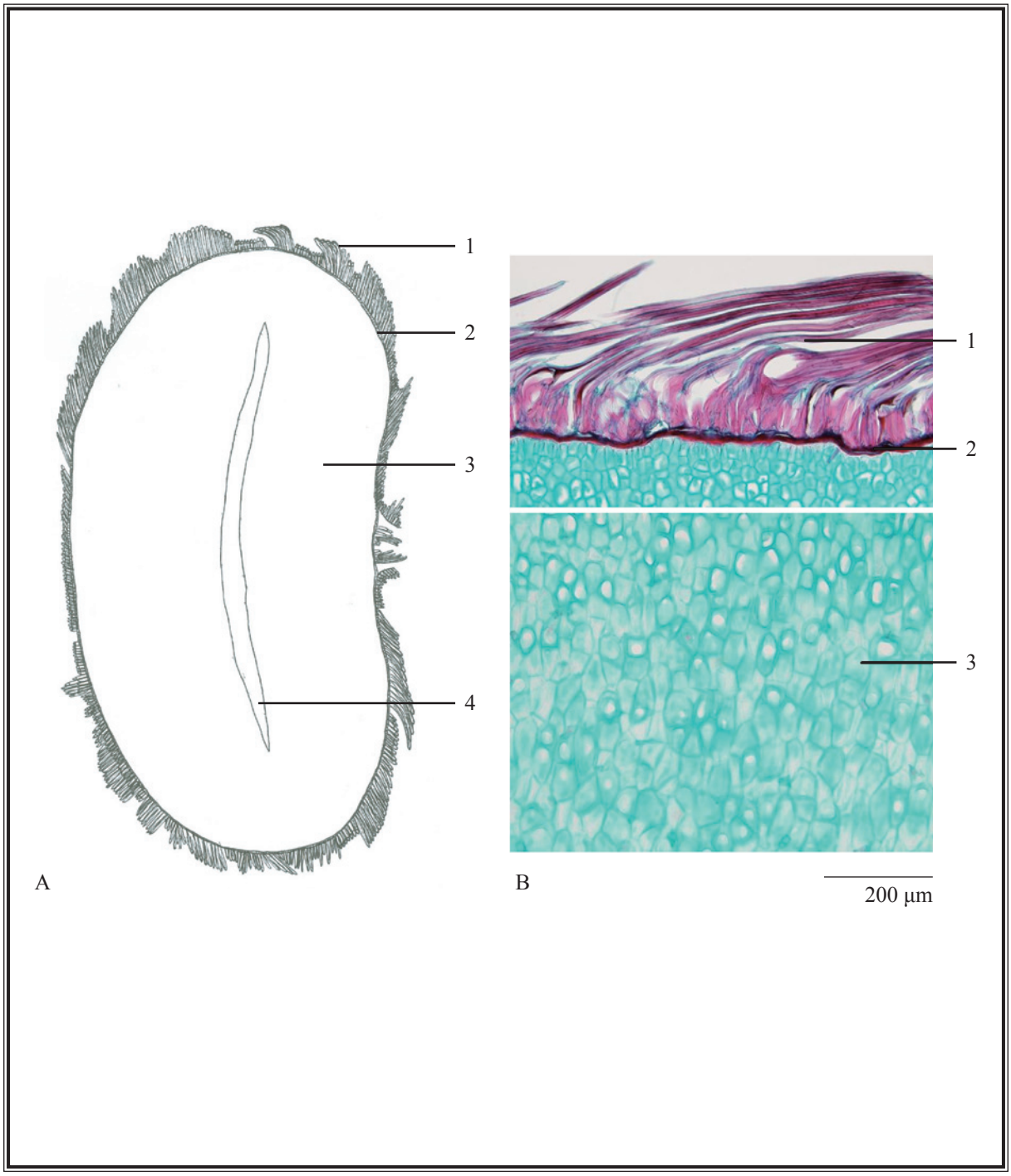


Figure 2 Microscopic features of transverse section of Strychni Semen (unprocessed)

A. Sketch B. Section illustration

1. Non-glandular hairs 2. Decadent parenchymatous cells 3. Endosperm 4. Cleft

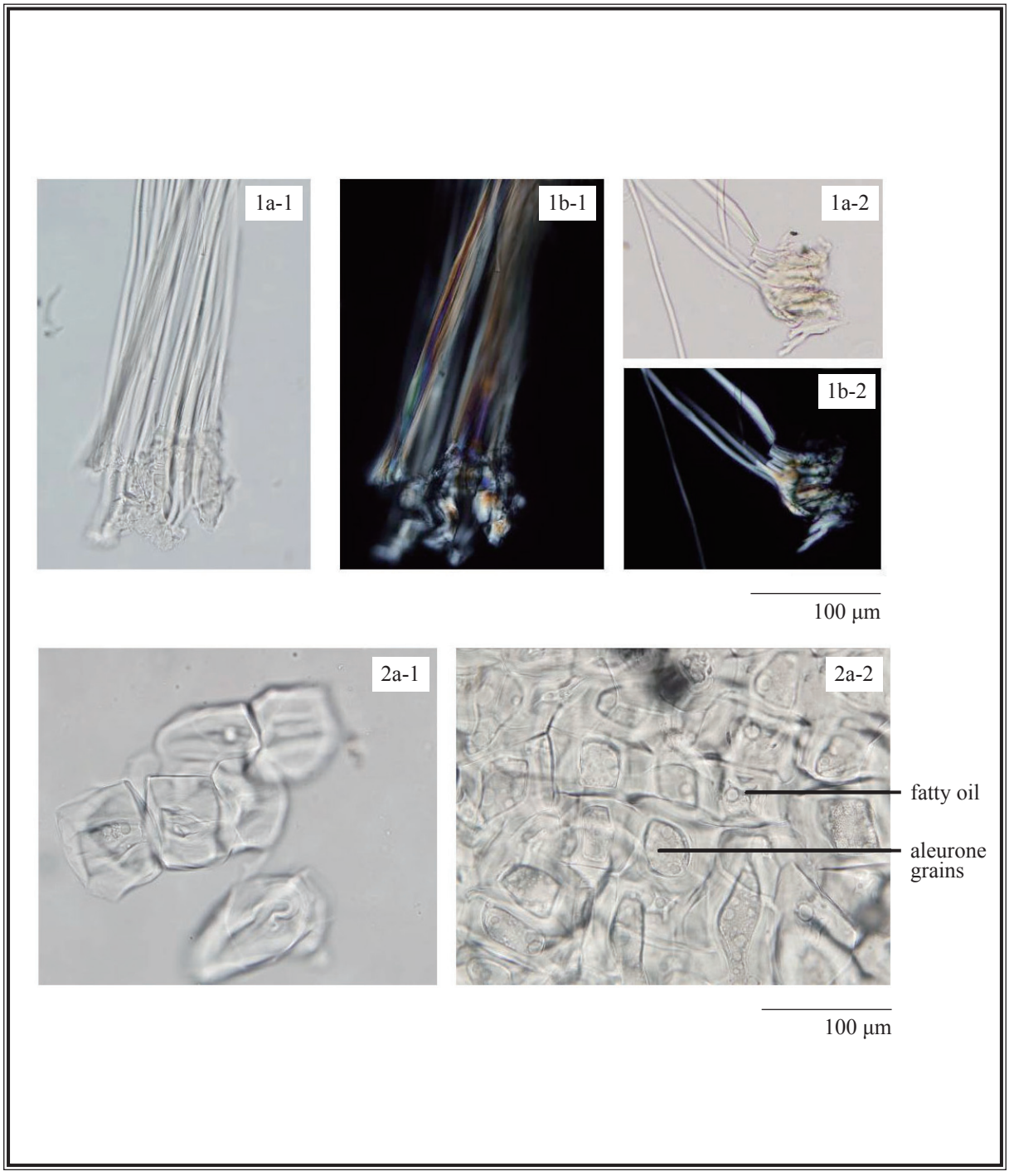


Figure 3 Microscopic features of powder of Strychni Semen (unprocessed)

1. Non-glandular hairs (1-1 non-glandular hairs, 1-2 base of non-glandular hairs)
2. Endosperm cells (2-1 endosperm cells, 2-2 endosperm cells containing fatty oil and aleurone grains)

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

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

Standard solutions

Brucine standard solution

Weigh 1.0 mg of brucine CRS (Fig. 4) and dissolve in 1 mL of diethyl ether.

Strychnine standard solution

Weigh 1.0 mg of strychnine CRS (Fig. 4) and dissolve in 1 mL of diethyl ether.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), isopropanol and ethyl acetate (0.8:2:7.5, 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.

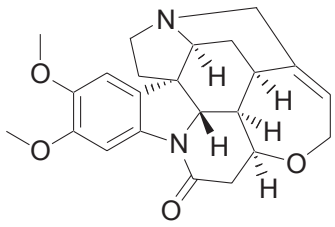
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 0.5 mL of ammonium hydroxide solution (9.1%, w/v) and 15 mL of diethyl ether. Cap the flask. Shake well for 5 min and allow to stand for 2 h. 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 brucine standard solution, strychnine standard solution and the test solution (10 µL each) to the plate. 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 visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

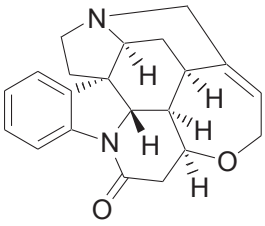


Figure 4 Chemical structures of (i) brucine and (ii) strychnine

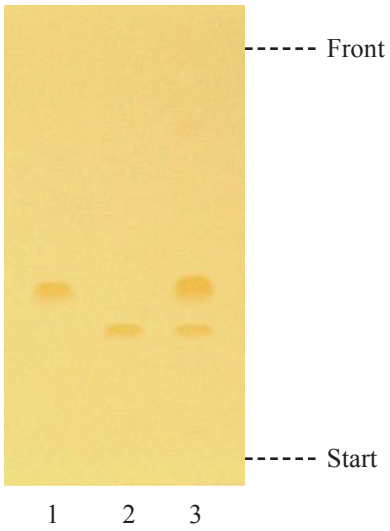


Figure 5 A reference HPTLC chromatogram of Strychni Semen (unprocessed) extract observed under visible light after staining

1. Strychnine standard solution 2. Brucine 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 brucine and strychnine (Fig. 5).

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

Reagents

0.01 M Sodium 1-heptanesulphonate solution

Weigh 1.01 g of sodium 1-heptanesulphonate and dissolve in 500 mL of water.

0.02 M potassium dihydrogen phosphate solution

Weigh 1.36 g of potassium dihydrogen phosphate and dissolve in 500 mL of water.

Sodium 1-heptanesulphonate – potassium dihydrogen phosphate buffer solution (pH 2.8)

Transfer 500 mL of 0.01 M sodium 1-heptanesulphonate solution and 500 mL of 0.02 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 2.8 with 10% phosphoric acid.

Standard solutions

Brucine standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of brucine CRS and dissolve in 10 mL of methanol.

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

Weigh 1.0 mg of strychnine CRS and dissolve in 5 mL of methanol.

Test solution

Weigh 0.6 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of a mixture of methanol, water and hydrochloric acid (50:50:1, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 5000 x *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 a mixture of methanol, water and hydrochloric acid (50:50:1, v/v). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 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)	Sodium 1-heptanesulphonate – potassium dihydrogen phosphate buffer solution (pH 2.8) (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	92	8	isocratic
5 – 15	92 → 80	8 → 20	linear gradient
15 – 50	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of brucine Std-FP and strychnine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of brucine and strychnine should not be more than 5.0%; the RSD of the retention times of brucine and strychnine peaks should not be more than 2.0%; the column efficiencies determined from brucine and strychnine peaks should not be less than 25000 theoretical plates.

The *R* value between peak 4 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 brucine Std-FP, strychnine Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of brucine and strychnine peaks in the chromatograms of brucine Std-FP, strychnine Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify brucine and strychnine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of brucine Std-FP and strychnine Std-FP. The retention times of brucine and strychnine 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 Strychni Semen (unprocessed) extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.36	± 0.03
2	0.41	± 0.04
3	0.58	± 0.06
4 (brucine)	0.91	± 0.03
5 (marker, strychnine)	1.00	-

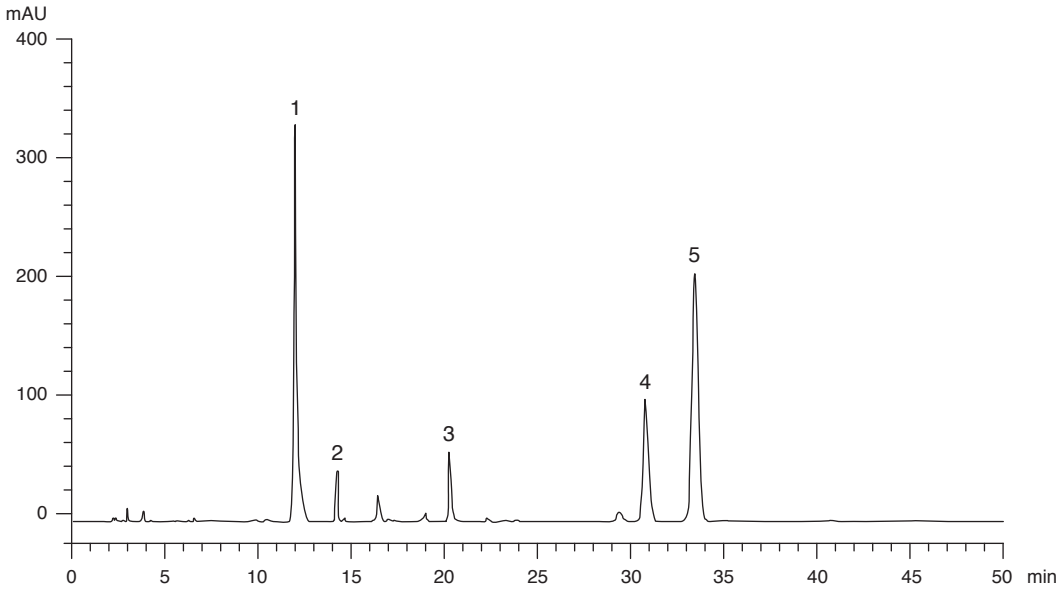


Figure 6 A reference fingerprint chromatogram of Strychni Semen (unprocessed) extract

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 – 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 1.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 13.0%.
Ethanol-soluble extractives (hot extraction method): not less than 11.0%.

7. ASSAY

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

Reagents

0.01 M Sodium 1-heptanesulphonate solution
Weigh 1.01 g of sodium 1-heptanesulphonate and dissolve in 500 mL of water.
0.02 M potassium dihydrogen phosphate solution
Weigh 1.36 g of potassium dihydrogen phosphate and dissolve in 500 mL of water.
Sodium 1-heptanesulphonate – potassium dihydrogen phosphate buffer solution (pH 2.8)
Transfer 500 mL of 0.01 M sodium 1-heptanesulphonate solution and 500 mL of 0.02 M potassium dihydrogen phosphate solution to a 1500-mL conical flask. Adjust the pH to 2.8 with 10% phosphoric acid.

Standard solution

Mixed brucine and strychnine standard stock solution, Std-Stock (250 mg/L for brucine and 500 mg/L for strychnine)
Weigh accurately 2.5 mg of brucine CRS and 5.0 mg of strychnine CRS, and dissolve in 10 mL of methanol.
Mixed brucine and strychnine standard solution for assay, Std-AS
Measure accurately the volume of the mixed brucine and strychnine Std-Stock, dilute with methanol to produce a series of solutions of 50, 75, 100, 125, 150 mg/L for brucine and 50, 100, 150, 200, 300 mg/L for strychnine.

Test solution

Weigh accurately 0.6 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of a mixture of methanol, water and hydrochloric acid (50:50:1, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifuge tube. Centrifuge at about 5000 x *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 a mixture of methanol, water and hydrochloric acid (50:50:1, v/v). Filter through a 0.45-μm nylon 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. The mobile phase is a mixture of sodium 1-heptanesulphonate – potassium dihydrogen phosphate buffer solution (pH 2.8) and acetonitrile (79:21, v/v). The elution time is about 26 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed brucine and strychnine Std-AS (100 mg/L for brucine and 150 mg/L for strychnine). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of brucine and strychnine should not be more than 5.0%; the RSD of the retention times of brucine and strychnine peaks should not be more than 2.0%; the column efficiencies determined from brucine and strychnine peaks should not be less than 10000 theoretical plates.

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

Limits

The sample contains 0.69% to 1.6% of brucine ($C_{23}H_{26}N_2O_4$) and 1.2% to 2.2% of strychnine ($C_{21}H_{22}N_2O_2$), calculated with reference to the dried substance.

8. CAUTION

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