Trigonellae Semen

Figure 1 A photograph of Trigonellae Semen

A. Seeds B. Magnified seeds C. Transverse section of seeds D. Cotyledons

1. NAMES

Official Name: Trigonellae Semen

Chinese Name: 胡蘆巴

Chinese Phonetic Name: Huluba

2. SOURCE

Trigonellae Semen is the dried ripe seed of *Trigonella foenum-graecum* L. (Fabaceae). The plant is collected in summer when the fruit is ripe. The fruit dried under the sun, the seed tapped out and foreign matter removed to obtain Trigonellae Semen.

3. DESCRIPTION

Slightly rhomboid or rectangular, 3-4 mm long, 2-3 mm wide, about 2 mm thick. Externally yellowish-green to yellowish-brown, smooth, with a deep oblique furrow on each of the two sides, and a dot-like hilum at the intersection of the two furrows. Texture hard, uneasily broken. Testa thin, endosperm translucent and viscous; cotyledons 2, pale yellow to brown, radicle curved, plump and long. Odour aromatic; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The outermost layer of the testa consists of palisade cells, covered with cuticle, thick wall, apex cusp, striations distinct. Radicle cells polygonal to subrounded. Beaker cells 1 layer, somewhat dumbbell-shaped, narrow at the upper end but broader at the lower end with indistinct linear striations on the anticlinal walls. Beneath beaker cells, 2-4 layers of parenchymatous cells visible. Aleurone layer located in the outer side of the endosperm, consisting of 1 layer of cells, the cells subsquare or elliptical. Endosperm mostly consists of mucilage cells. Cotyledon cells polygonal or elongated in transverse section view.

Powder

Colour pale yellow to brown. Palisade cells of testa with fine and close longitudinal pit canals in lateral view, polygonal in surface view, 40-85 μm long, wall thickened, lumen small. Beaker cells somewhat dumbbell-shaped, narrow at the upper end but broader at lower end with linear striations on anticlinal walls in lateral view; subrounded or polygonal, with dense radial striations in surface view. Parenchymatous cells and aleurone grains sometimes visible in seed fragments. Cotyledon cells oval or elongated, containing aleurone grains and oil droplets (Fig. 3).

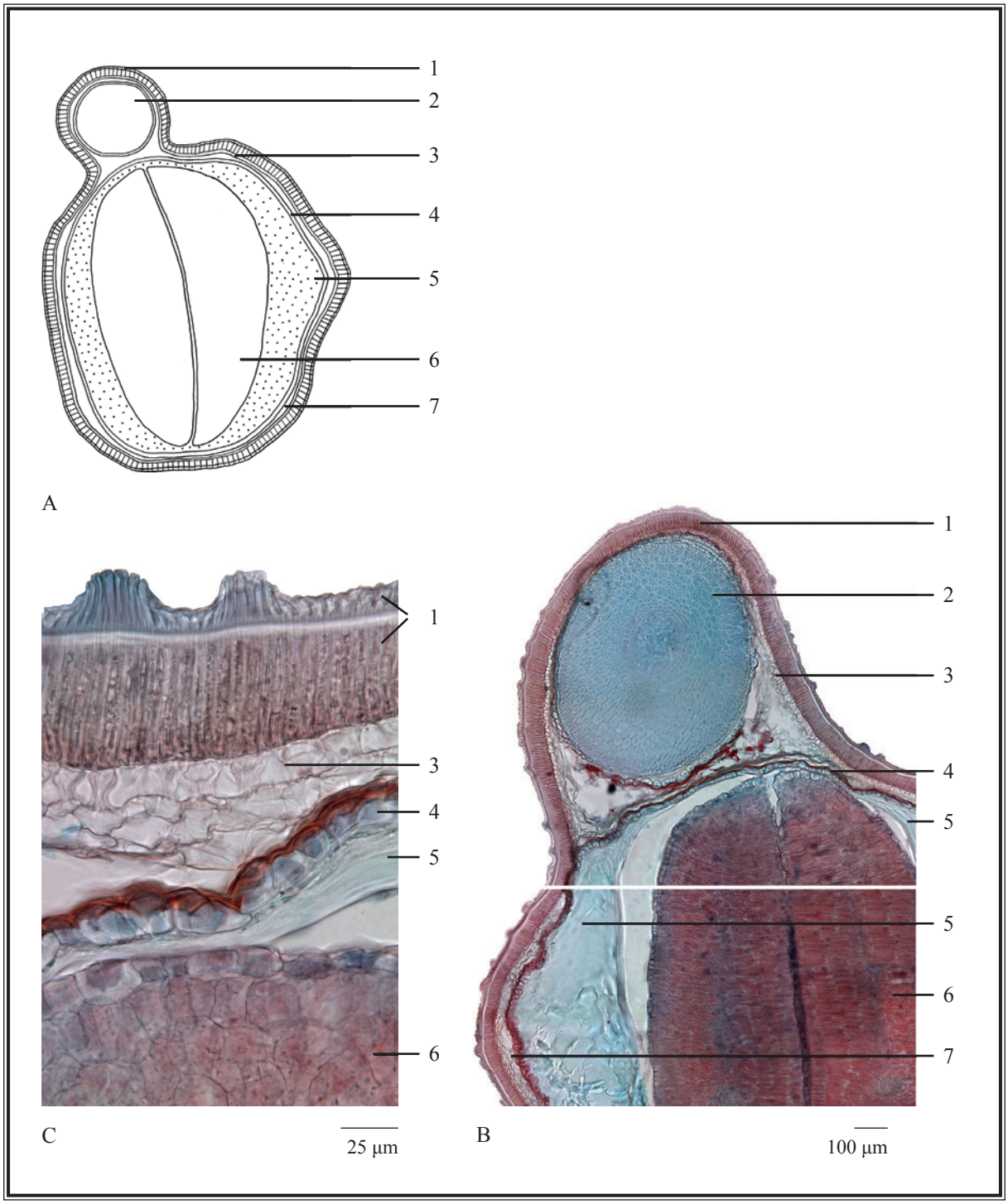

Figure 2 Microscopic features of transverse section of Trigonellae Semen

A. Sketch B. Section illustration C. Part of section magnified

1. Palisade cells of testa 2. Radicle 3. Beaker cells
4. Aleurone layer 5. Endosperm 6. Cotyledon 7. Parenchyma

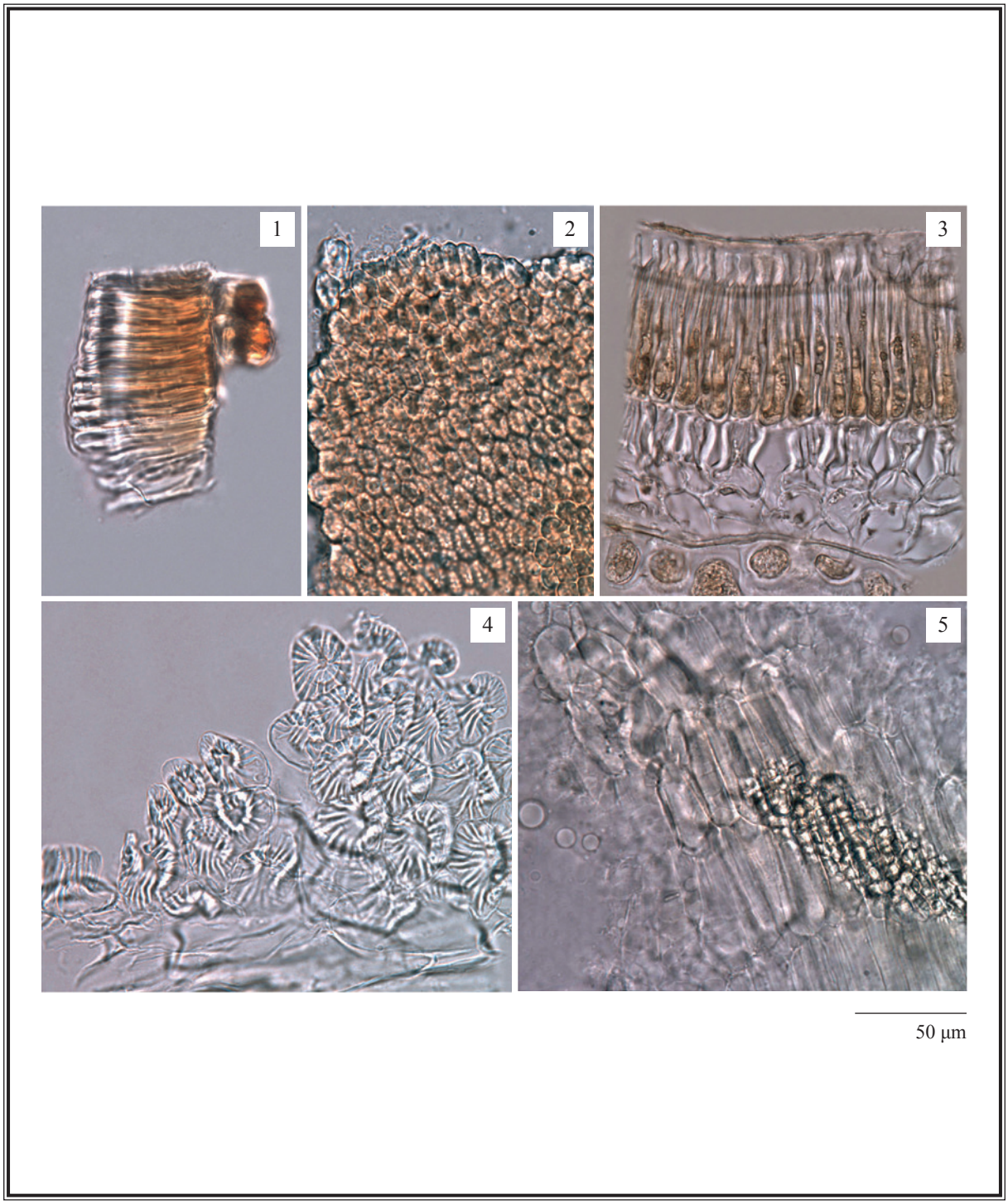

Figure 3 Microscopic features of powder of Trigonellae Semen (under the light microscope)

1. Palisade cells of testa (in lateral view) 2. Palisade cells of testa (in surface view)
3. A fragment of the seed's outer portion, showing cell types of the palisade layer, beaker layer, parenchymatous layer and aleurone layer
4. Beaker cells (in surface view) 5. Cotyledon cells with aleurone grains and fatty oil droplets

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

Standard solution

Trigonelline standard solution

Weigh 1.0 mg of trigonelline CRS (Fig. 4) and dissolve in 0.5 mL of ethanol.

Developing solvent system

Prepare a mixture of n-butanol, hydrochloric acid and ethyl acetate (8:3:1, 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. Place it in a water bath at about 50°C to dissolve the mixture.

Solution B

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

Solution C

Transfer 1 mL of Solution A, 1 mL of Solution B and 4 mL of glacial acetic acid into a 20-mL volumetric flask and make up to the mark with water.

Solution D

Weigh 4.5 g of ferric trichloride and dissolve in 50 mL of water.

Spray reagent

Mix 10 mL of Solution C and 5 mL of Solution D in a 50-mL conical flask. Freshly prepare all the reagents.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of petroleum ether (60-80°C). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Discard the supernatant. Add 30 mL of ethanol to the residue. Sonicate (160 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol.

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 trigonelline standard solution (3 µL) and the test solution (5 µL) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Heat the plate at about 105˚C for 1 h. Cool down to room temperature. Spray the plate evenly with the spray reagent

and dry it in air until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of trigonelline.

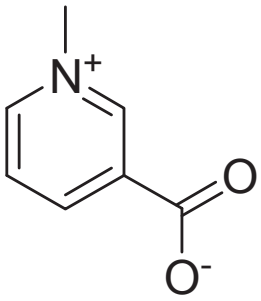

Figure 4 Chemical structure of trigonelline

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

Standard solution

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

Weigh 1.0 mg of trigonelline CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC 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 flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 40	95 → 71	5 → 29	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of trigonelline Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of trigonelline should not be more than 5.0%; the RSD of the retention time of trigonelline peak should not be more than 2.0%; the column efficiency determined from trigonelline peak should not be less than 5000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1 (marker, trigonelline)	1.00	-
2	8.93	± 0.05
3	10.78	± 0.03
4	11.02	± 0.03

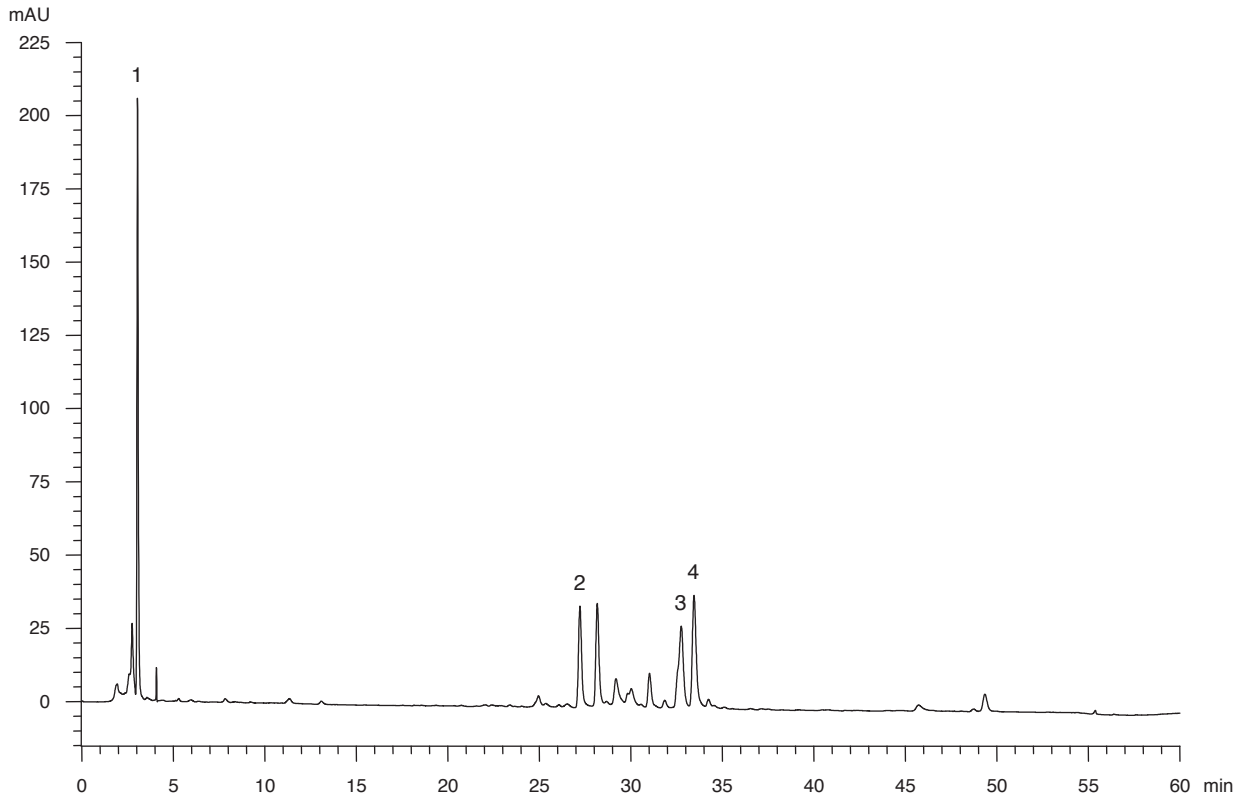

Figure 5 A reference fingerprint chromatogram of Trigonellae Semen extract

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 *(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 4.0%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Ethanol-soluble extractives (cold extraction method): not less than 18.0%.

7. ASSAY

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

Standard solution

Trigonelline standard stock solution, Std-Stock (300 mg/L)

Weigh accurately 3.0 mg of trigonelline CRS and dissolve in 10 mL of ethanol (50%).

Trigonelline standard solution for assay, Std-AS

Measure accurately the volume of the trigonelline Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1.5, 30, 60, 100, 150 mg/L for trigonelline.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (160 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a Hydrophilic Interaction Chromatography (HILIC) column (4.6 × 250 mm), 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)	0.1% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	30 → 40	70 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of trigonelline Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of trigonelline should not be more than 5.0%; the RSD of the retention time of trigonelline peak should not be more than 2.0%; the column efficiency determined from trigonelline peak should not be less than 10000 theoretical plates.

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

Calibration curve

Inject a series of trigonelline Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of trigonelline against the corresponding concentrations of trigonelline Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify trigonelline peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of trigonelline Std-AS. The retention times of trigonelline peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of trigonelline in the test solution, and calculate the percentage content of trigonelline in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.45% of trigonelline ($C_7H_7NO_2$), calculated with reference to the dried substance.