

Polygonati Rhizoma

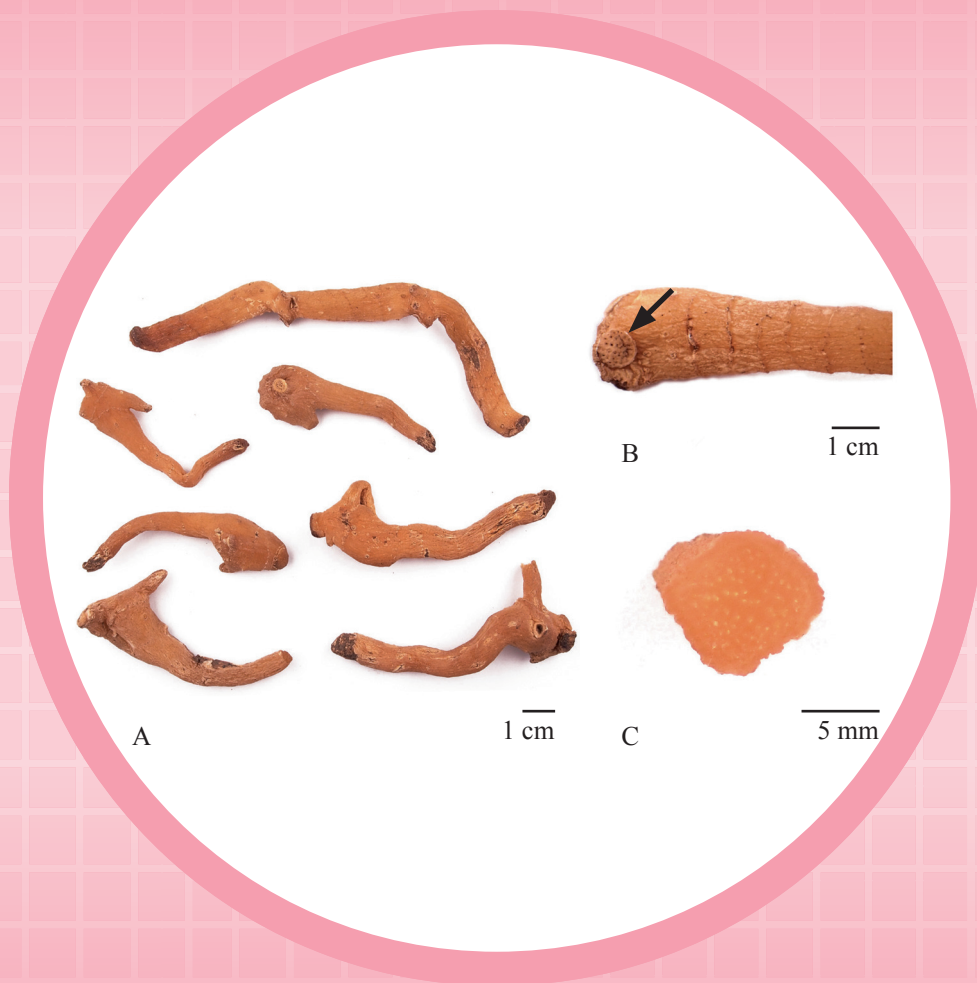


Figure 1 A photograph of Polygonati Rhizoma

- A. Polygonati Rhizoma B. Magnified image of rhizome (stem scar →)
C. Magnified image of fracture surface

1. NAMES

Official name: Polygonati Rhizoma

Chinese name: 黃精

Chinese phonetic name: Huangjing

2. SOURCE

Polygonati Rhizoma is the dried rhizome of *Polygonatum sibiricum* Red. (Liliaceae). The rhizome is collected in autumn, foreign matter and fibrous roots removed, washed clean, steamed thoroughly, or immersed in boiling water for a moment, then dried under the sun or baked to dryness to obtain Polygonati Rhizoma.

3. DESCRIPTION

Elongated, nodular, varying in length, 2.7-15.2 cm long, 3-25 mm in diameter, often tapered at one end, branched, with protruded yellowish-white stem scars. Externally yellowish, greyish-yellow to yellowish-brown, with nodes, sometimes indistinct, internode 1-14 mm long. Heavy in weight, texture hard, fracture flat, horny, yellowish-white to yellowish-brown, slightly translucent, with numerous yellowish-white vein dots scattered. Odour slight; taste slightly sweet (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Epidermis consists of 1 layer of cells, covered with cuticle. Cortex relatively narrow. Stele broad, endodermis indistinct, with vascular bundles scattered; vascular bundles mainly collateral, relatively smaller and denser on the outer side. Mucilage cells scattered in parenchyma, some containing raphides of calcium oxalate, relatively denser on the outer side (Fig. 2).

Powder

Colour yellowish-brown. Raphides of calcium oxalate in bundles; scattered or present in mucilage cells, 30-220 µm in diameter; polychromatic under the polarized microscope. Epidermal cells subpolygonal in surface view, anticlinal walls slightly beaded, covered with yellow cuticle. Vessels mainly scalariform and reticulate, 8-70 µm in diameter. Parenchymatous cells numerous, large, subpolygonal or subrounded, some with faintly visible pits (Fig. 3).

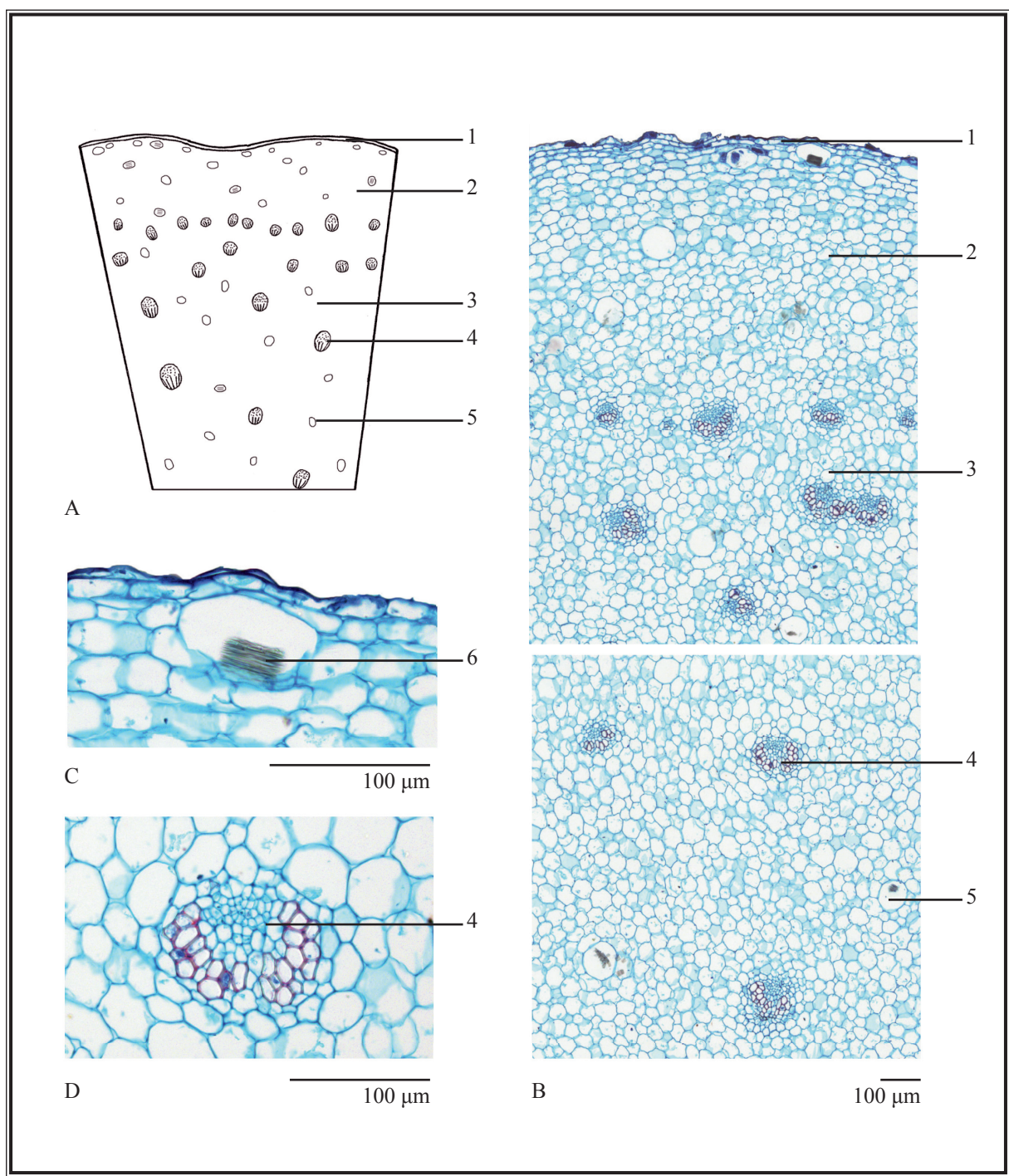


Figure 2 Microscopic features of transverse section of Polygonati Rhizoma

A. Sketch B. Section illustration C. Mucilage cell D. Vascular bundle

1. Epidermis 2. Cortex 3. Stele 4. Vascular bundle 5. Mucilage cell
 6. Raphides of calcium oxalate

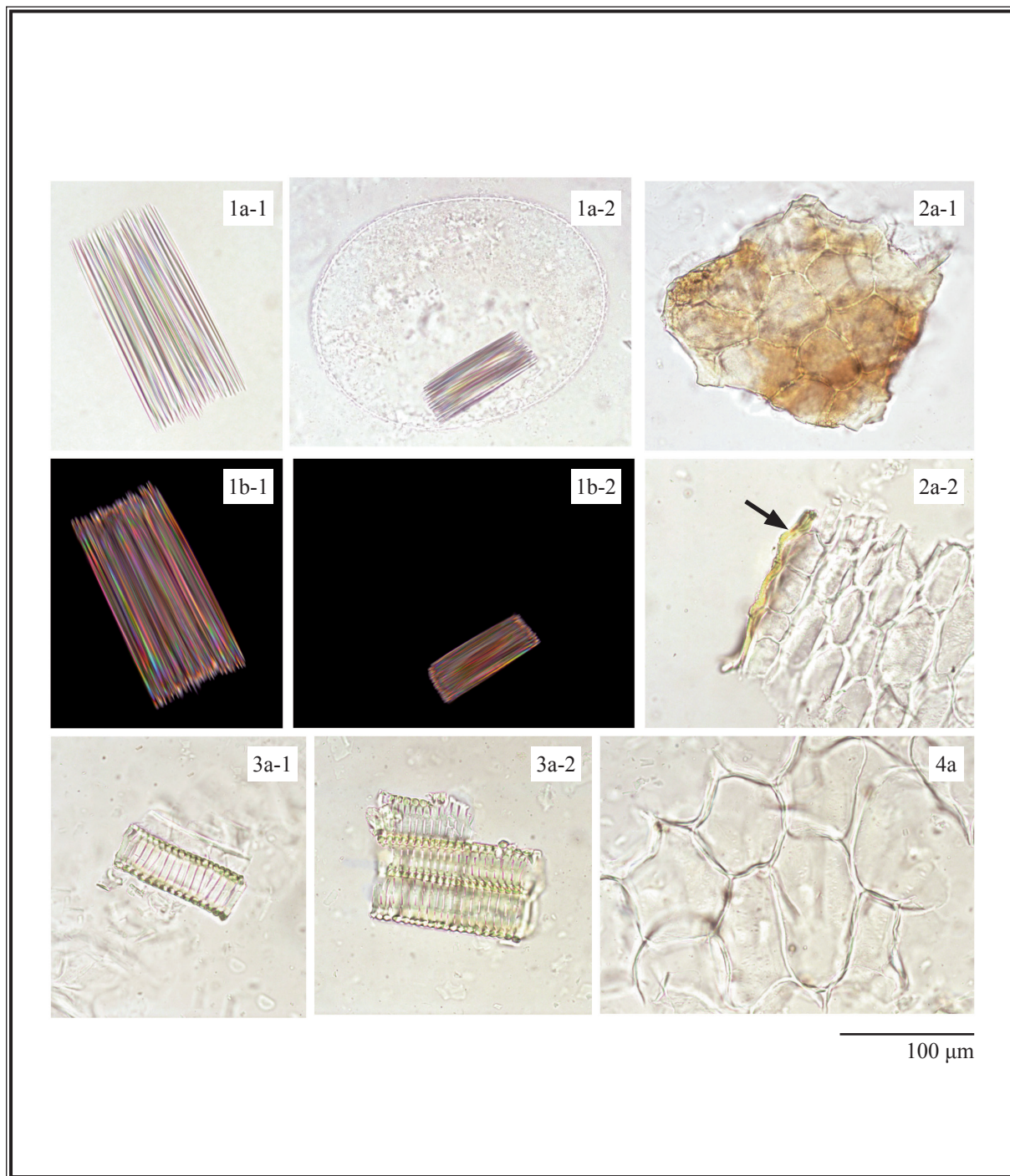


Figure 3 Microscopic features of powder of *Polygonati Rhizoma*

1. Raphides of calcium oxalate (1-1 scattered, 1-2 in mucilage cell)
2. Epidermal cells (2-1 surface view, 2-2 lateral view) (cuticle →)
3. Vessels (3-1 scalariform vessel, 3-2 reticulate vessels) 4. Parenchymatous cells

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

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 1 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of *n*-butanol, formic acid and ethyl acetate (8: 8: 1, v/v).

Staining reagent

Iodine.

Test solution

Weigh 2.0 g of the powdered sample (through a No. 5 sieve) and place it in a 100-mL round-bottomed flask, then add 50 mL of methanol with 0.1% formic acid. Reflux the mixture for 45 min. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction one more time. Combine the filtrates. Evaporate the combined filtrates to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 10-mL volumetric flask and make up to the mark with ethanol (50%). Pre-condition a solid phase extraction (SPE) column (Neutral aluminium oxide, 20 mL, 5 g) with 20 mL of ethanol (50%). Load 5 mL of the extract to the pre-conditioned SPE column. Add 50 mL of ethanol (50%) to the column. Collect the eluate and transfer to a 100-mL round-bottomed flask. Evaporate the eluate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.22- μ m nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Apply separately trigonelline standard solution (2 μ L) and the test solution (5 μ L) 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. Expose the plate to iodine vapour in a chamber for about 20 min 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).

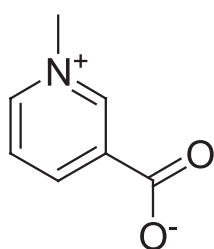


Figure 4 Chemical structure of trigonelline

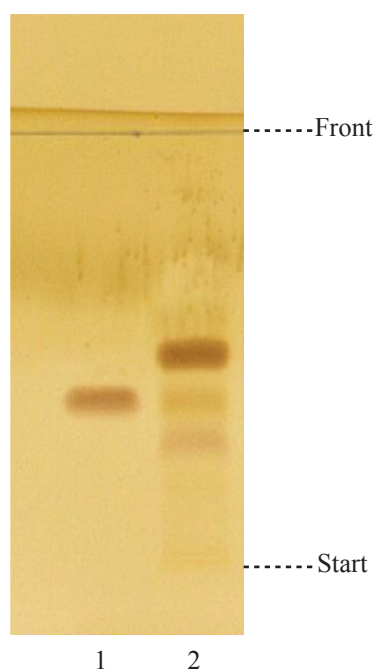


Figure 5 A reference HPTLC chromatogram of *Polygonati Rhizoma* extract observed under visible light after staining

1. Trigonelline standard solution 2. Test solution

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 (Fig. 5).

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

Reagents

0.2M Ammonium acetate solution

Weigh 7.71 g of ammonium acetate and dissolve in 500 mL water.

0.01M Ammonium acetate solution

Pipette 50 mL of 0.2M ammonium acetate solution and make up to 1000 mL of water.

0.2% Acetic acid in 0.01M ammonium acetate solution

Pipette 2 mL of acetic acid into 1000 mL of 0.01M ammonium acetate solution.

Mixture of acetic acid and 0.2M ammonium acetate in acetonitrile

Pipette 2 mL of acetic acid into 50 mL of 0.2M ammonium acetate solution. Add the solution of acetic acid in ammonium acetate into 950 mL of acetonitrile.

Standard solution

Trigonelline standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 2.5 mg of trigonelline CRS and dissolve in 100 mL of ethanol (50%).

Test solution

Weigh 2.0 g of the powdered sample (through a No. 5 sieve) and place it in a 100-mL round-bottomed flask, then add 50 mL of methanol with 0.1% formic acid. Reflux the mixture for 45 min. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction two more times. Combine the filtrates. Evaporate the combined filtrates to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 10-mL volumetric flask and make up to the mark with ethanol (50%). Pre-condition a solid phase extraction (SPE) column (Neutral aluminium oxide, 20 mL, 5 g) with 20 mL of ethanol (50%). Load 5 mL of the extract to the pre-conditioned SPE column. Add 50 mL of ethanol (50%) to the column. Collect the eluate and transfer to a 100-mL round-bottomed flask. Evaporate the eluate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.22- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a Hydrophilic Interaction Chromatography (HILIC) column (2.1 \times 150 mm, 1.7 μ m particle size, 130 Å pore size and 185 m²/g surface area). The column temperature is maintained at 40°C during the separation. The flow rate is about 0.4 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Mixture of acetic acid and 0.2M ammonium acetate in acetonitrile (% v/v)	0.2% Acetic acid in 0.01M ammonium acetate solution (% v/v)	Elution
0 – 3	100 → 93	0 → 7	linear gradient
3 – 10	93 → 88	7 → 12	linear gradient
10 – 15	88	12	isocratic

System suitability requirements

Perform at least five replicate injections, each using 1 μ L 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 80000 theoretical plates.

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. 6).

Procedure

Separately inject trigonelline Std-FP and the test solution (1 μ L each) into the UHPLC 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 three characteristic peaks (Fig. 6) 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 three characteristic peaks of *Polygonati Rhizoma* extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of *Polygonati Rhizoma* extract

Peak No.	RRT	Acceptable Range
1	0.35	± 0.03
2	0.38	± 0.03
3 (marker, trigonelline)	1.00	-

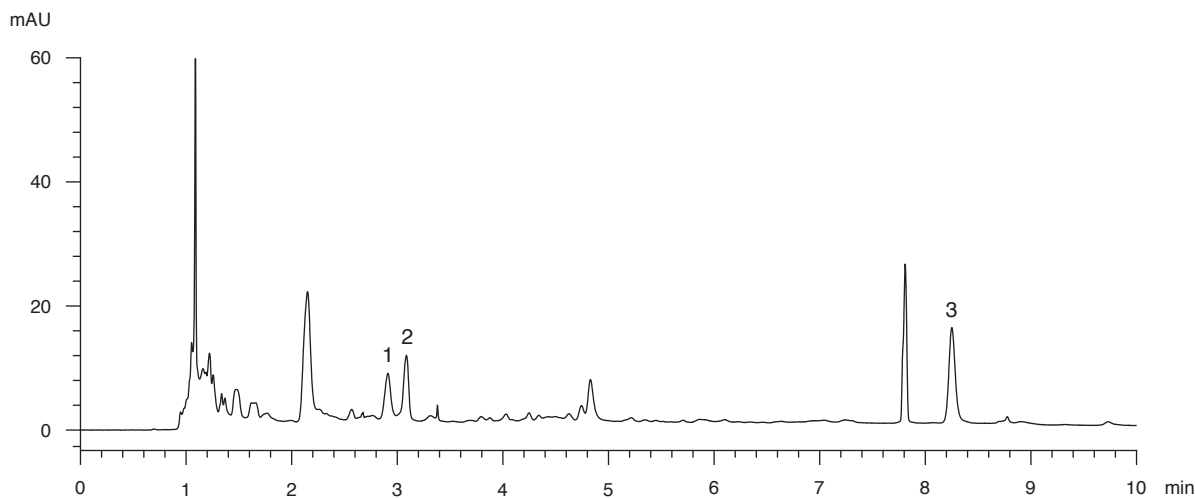


Figure 6 A reference fingerprint chromatogram of Polygonati Rhizoma extract

For positive identification, the sample must give the above three 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 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 3.0%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*)

Toluene distillation method: not more than 11.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 63.0%.

Ethanol-soluble extractives (cold extraction method): not less than 64.0%.

7. ASSAY

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

Reagents

0.2M Ammonium acetate solution

Weigh 7.71 g of ammonium acetate and dissolve in 500 mL water.

0.01M Ammonium acetate solution

Pipette 50 mL of 0.2M ammonium acetate solution and make up to 1000 mL of water.

0.2% Acetic acid in 0.01M ammonium acetate solution

Pipette 2 mL of acetic acid into 1000 mL of 0.01M ammonium acetate solution.

Mixture of acetic acid and 0.2M ammonium acetate in acetonitrile

Pipette 2 mL of acetic acid into 50 mL of 0.2M ammonium acetate solution. Add the solution of acetic acid in ammonium acetate into 950 mL of acetonitrile.

Standard solution

Trigonelline standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 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 3, 5, 10, 25, 50 mg/L for trigonelline.

Test solution

Weigh accurately 2.0 g of the powdered sample (through a No. 5 sieve) and place it in a 100-mL round-bottomed flask, then add 50 mL of methanol with 0.1% formic acid. Reflux the mixture for 45 min. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction two more times. Combine the filtrates. Evaporate the combined filtrates to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 10-mL volumetric flask and make up to the mark with ethanol (50%). Pre-condition a solid phase extraction (SPE) column (Neutral aluminium oxide, 20 mL, 5 g) with 20 mL of ethanol (50%). Load 5 mL of the extract to the pre-conditioned SPE column. Add 50 mL of ethanol (50%) to the column. Collect the eluate and transfer to a 100-mL round-bottomed flask. Evaporate the eluate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (50%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.22- μ m nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a Hydrophilic Interaction Chromatography (HILIC) column (2.1 \times 150 mm, 1.7 μ m particle size, 130 Å pore size and 185 m²/g surface area). The column temperature is maintained at 40°C during the separation. The flow rate is about 0.4 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Mixture of acetic acid and 0.2M ammonium acetate in acetonitrile (% v/v)	0.2% Acetic acid in 0.01M ammonium acetate solution (% v/v)	Elution
0 – 3	100 → 93	0 → 7	linear gradient
3 – 10	93 → 88	7 → 12	linear gradient
10 – 15	88	12	isocratic

System suitability requirements

Perform at least five replicate injections, each using 1 μ L of trigonelline Std-AS (10 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 80000 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 (Fig. 7).

Calibration curve

Inject a series of trigonelline Std-AS (1 μ L each) into the UHPLC 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 1 μ L of the test solution into the UHPLC system and record the chromatogram. Identify trigonelline peak (Fig. 7) 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 0.0034% of trigonelline ($C_7H_7NO_2$), calculated with reference to the dried substance.

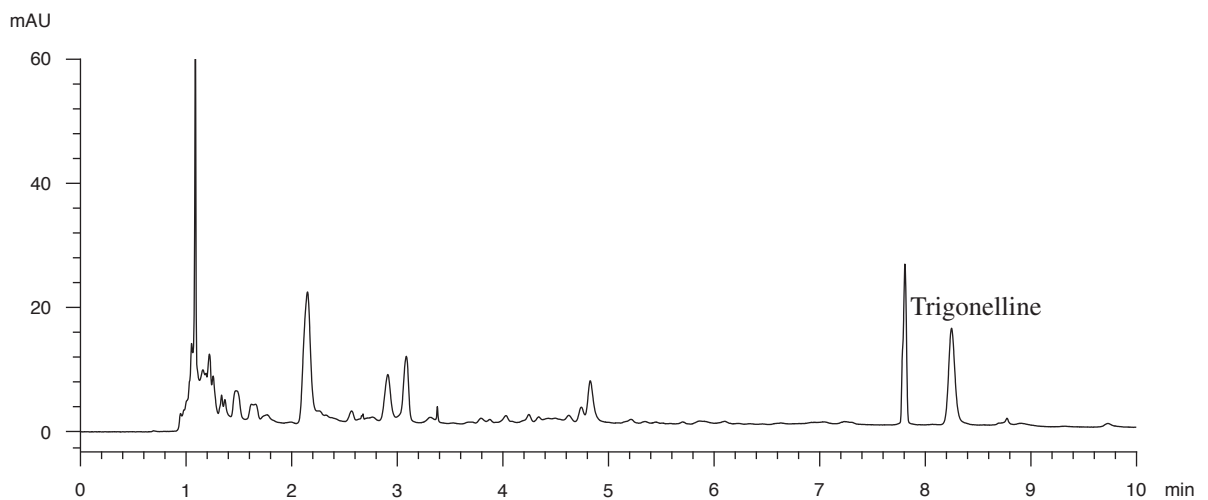


Figure 7 A reference assay chromatogram of Polygonati Rhizoma extract