Sennae Folium

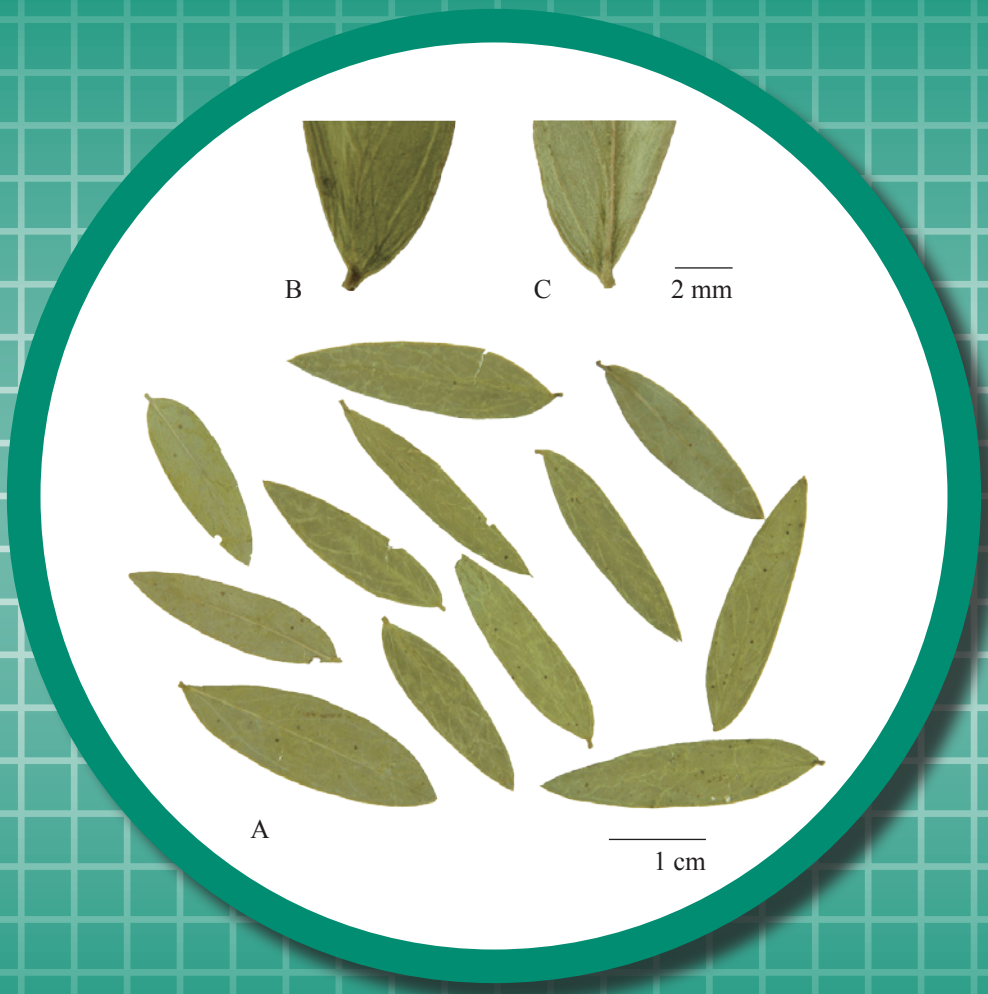

Figure 1 A photograph of Sennae Folium

A. Sennae Folium B. Magnified upper surface of leaflet
C. Magnified lower surface of leaflet

1. NAMES

Official Name: Sennae Folium

Chinese Name: 番瀉葉

Chinese Phonetic Name: Fanxieye

2. SOURCE

Sennae Folium is the dried leaflet of *Cassia angustifolia* Vahl (Fabaceae). The leaflet is collected before flowering, then dried in a shaded area or baked at temperature about 40-50ºC to dryness to obtain Sennae Folium.

3. DESCRIPTION

Elongated ovate to ovate-lanceolate, 1.5-5 cm long, 0.4-2 cm wide, apex acute, base slightly asymmetric, margins entire. The upper surface yellowish-green; the lower surface pale yellowish-green, glabrous or nearly glabrous, veins slightly protuberant. Texture leathery. Odour slight and characteristic; taste slightly bitter, with slight viscousness (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Both upper epidermis and lower epidermis consist of 1 layer of epidermal cells with stomata. Mesophyll isobilateral, consisting of 2 layers of palisade tissue, located at inner side of upper epidermis and lower epidermis respectively. The upper layer cells of palisade tissue relative long, up to 150 μm long. The lower layer cells of palisade tissue 35-60 μm long. Prisms of calcium oxalate and clusters of calcium oxalate scattered in parenchymatous cells. Several dozens fibres in bundles, surrounded by parenchymatous cells containing prisms of calcium oxalate, forming crystal fibres. Vascular bundles collateral. Collenchyma located at the inner sides of the lower epidermis. Non-glandular hairs sometimes visible in lower epidermis (Fig. 2).

Powder

Colour pale green to yellowish-green. Epidermal cells polygonal in surface view, stomata present on both surfaces, mostly paracytic, subsidiary cells mostly 2, sometimes 3. Non-glandular hairs sometimes visible, unicellular, papillose. Crystal fibres numerous; polychromatic under the polarized microscope. Prisms of calcium oxalate 6-18 μm in diameter; polychromatic under the polarized microscope. Clusters of calcium oxalate present in parenchymatous cells of mesophyll, 9-23 μm in diameter; polychromatic under the polarized microscope (Fig. 3).

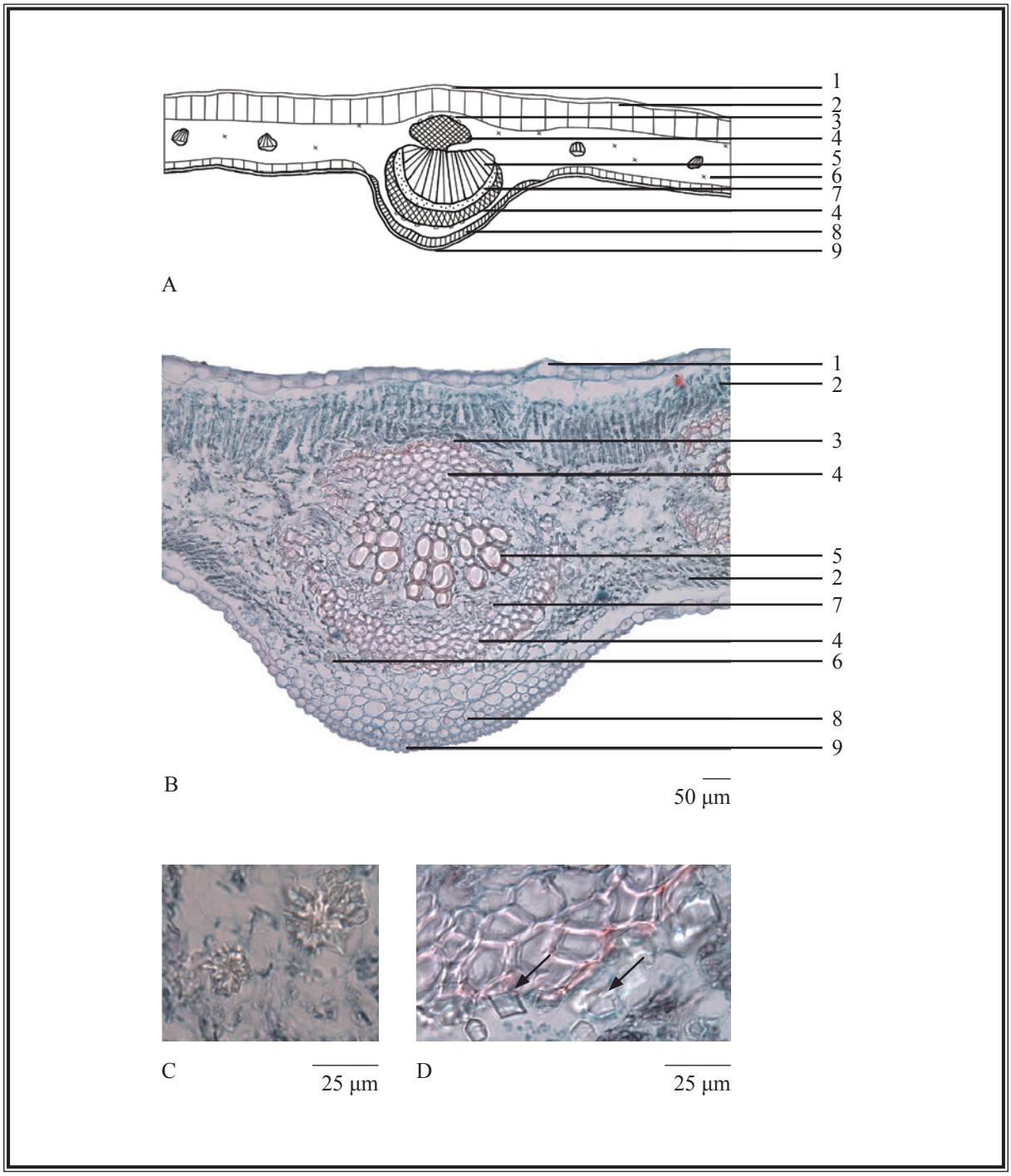

Figure 2 Microscopic features of transverse section of Sennae Folium

A. Sketch B. Section illustration C. Clusters of calcium oxalate D. Prisms of calcium oxalate

1. Upper epidermis 2. Palisade tissue 3. Prisms of calcium oxalate
4. Fibre bundle 5. Xylem 6. Clusters of calcium oxalate
7. Phloem 8. Collenchyma 9. Lower epidermis

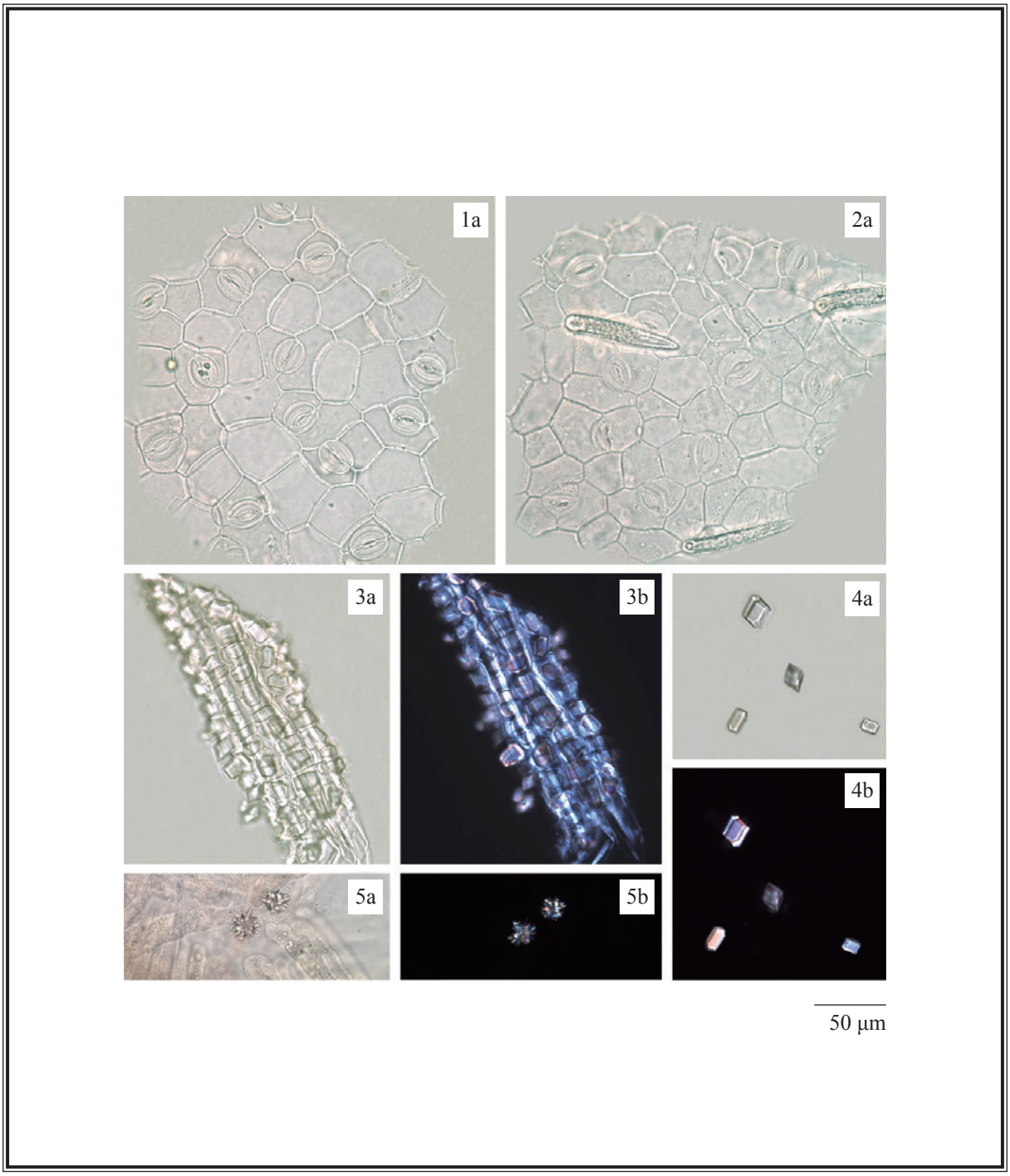

Figure 3 Microscopic features of powder of Sennae Folium

1. Upper epidermal cells with stomata 2. Lower epidermal cells with stomata and non-glandular hairs
3. Crystal fibres 4. Prisms of calcium oxalate 5. Clusters of calcium oxalate

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

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

Standard solutions

Sennoside A standard solution

Weigh 1.0 mg of sennoside A CRS (Fig. 4) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with ethanol (50%).

Sennoside B standard solution

Weigh 1.0 mg of sennoside B CRS (Fig. 4) and place it in a 1-mL amber glass volumetric flask. Make up to the mark with ethanol (50%).

Developing solvent system

Prepare a mixture of *n*-propanol, ethyl acetate, water and glacial acetic acid (3:3:2:0.2, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (50%). Sonicate (270 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 5 mL of ethanol (50%).

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately sennoside A standard solution, sennoside B standard solution and the test solution (1 µL 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 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

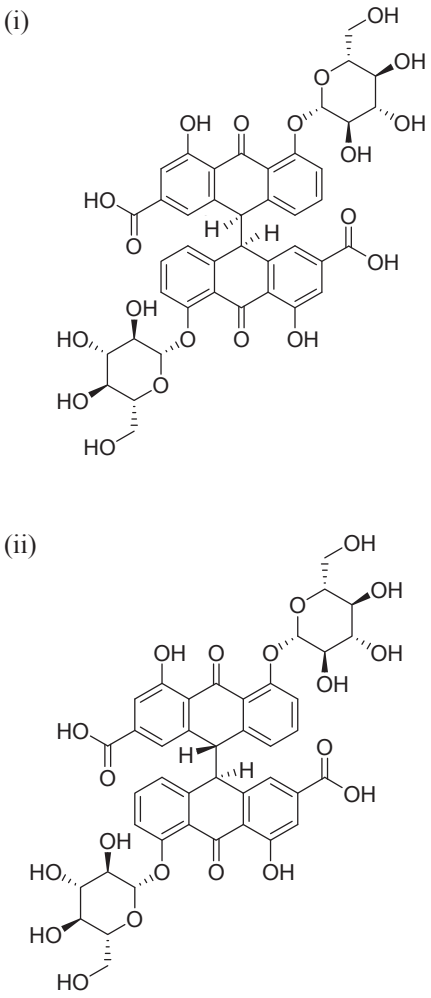

Figure 4 Chemical structures of (i) sennoside A and (ii) sennoside B

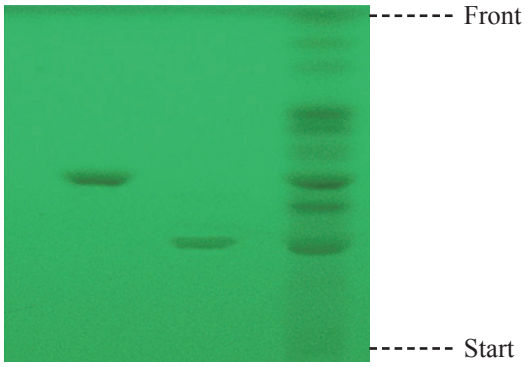

Figure 5 A reference HPTLC chromatogram of Sennae Folium extract observed under UV light (254 nm)

1. Sennoside A standard solution 2. Sennoside B 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 sennoside A and sennoside B (Fig. 5).

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

Standard solutions

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

Weigh 2.0 mg of sennoside A CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with sodium bicarbonate solution (0.1%, w/v).

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

Weigh 2.0 mg of sennoside B CRS and place it in a 10-mL amber glass volumetric flask. Make up to the mark with sodium bicarbonate solution (0.1%, w/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 10 mL of sodium bicarbonate solution (0.1%, w/v). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL amber glass volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with sodium bicarbonate solution (0.1%, w/v). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 45	85 → 75	15 → 25	linear gradient
45 – 60	75 → 10	25 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of sennoside A Std-FP and sennoside B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of sennoside A and sennoside B should not be more than 5.0%; the RSD of the retention times of sennoside A and sennoside B peaks should not be more than 2.0%; the column efficiencies determined from sennoside A and sennoside B peaks should not be less than 50000 and 30000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 6 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject sennoside A Std-FP, sennoside B Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of sennoside A and sennoside B peaks in the chromatograms of sennoside A Std-FP, sennoside B Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify sennoside A and sennoside B peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of sennoside A Std-FP and sennoside B Std-FP. The retention times of sennoside A and sennoside B 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 six characteristic peaks of Sennae Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Sennae Folium extract

Peak No.	RRT	Acceptable Range
1	0.33 (vs peak 3)	± 0.03
2	0.38 (vs peak 3)	± 0.03
3 (marker 1, sennoside B)	1.00	-
4	1.18 (vs peak 3)	± 0.03
5	1.22 (vs peak 3)	± 0.03
6 (marker 2, sennoside A)	1.00	-

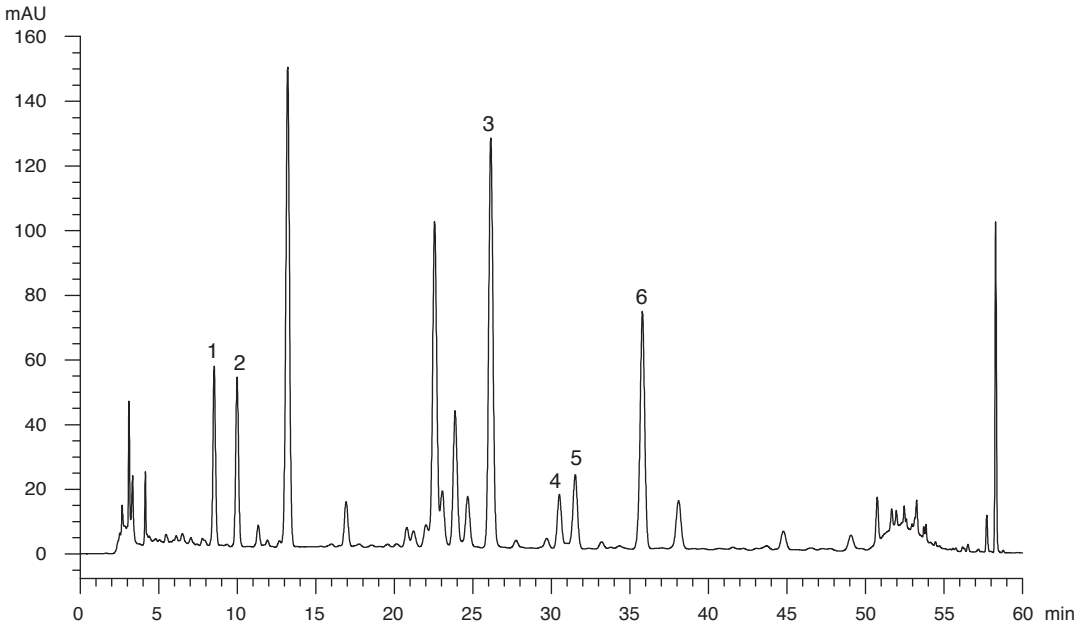

Figure 6 A reference fingerprint chromatogram of Sennae Folium extract

For positive identification, the sample must give the above six 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 4.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 25.0%.
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

Mixed sennoside A and sennoside B standard stock solution, Std-Stock (800 mg/L each)

Weigh accurately 4.0 mg of sennoside A CRS and 4.0 mg of sennoside B CRS, and place it in a 5-mL amber glass volumetric flask. Make up to the mark with sodium bicarbonate solution (0.1%, w/v).

Mixed sennoside A and sennoside B standard solution for assay, Std-AS

Measure accurately the volume of the mixed sennoside A and sennoside B Std-Stock, dilute with sodium bicarbonate solution (0.1%, w/v) to produce a series of solutions of 0.5, 50, 100, 200, 300 mg/L for both sennoside A and sennoside B.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube wrapped in aluminium foil, then add 10 mL of sodium bicarbonate solution (0.1%, w/v). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL amber glass volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with sodium bicarbonate solution (0.1%, w/v). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 45	85 → 75	15 → 25	linear gradient
45 – 60	75 → 10	25 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed sennoside A and sennoside B Std-AS (100 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of sennoside A and sennoside B should not be more than 5.0%; the RSD of the retention times of sennoside A and sennoside B peaks should not be more than 2.0%; the column efficiencies determined from sennoside A and sennoside B peaks should not be less than 50000 and 30000 theoretical plates respectively.

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

Limits

The sample contains not less than 1.1% of the total content of sennoside A ($C_{42}H_{38}O_{20}$) and sennoside B ($C_{42}H_{38}O_{20}$), calculated with reference to the dried substance.