Impatientis Caulis

Figure 1 A photograph of Impatientis Caulis

A. Impatientis Caulis
B. Magnified image of stem (showing longitudinal wrinkles and nodes)
C. Magnified image of transverse sections of stem

1. NAMES

Official name: Impatientis Caulis

Chinese name: 鳳仙透骨草

Chinese phonetic name: Fengxiantougucao

2. SOURCE

Impatientis Caulis is the dried stem of *Impatiens balsamina* L. (Balsaminaceae). The stem is collected in summer and autumn, foreign matter removed, then dried under the sun to obtain Impatientis Caulis.

3. DESCRIPTION

Long, tubular stem, with few branches, varying in length, up to 60 cm long, 3-20 mm in diameter. Externally pale yellowish-white, yellowish-brown to reddish-brown, shriveled, longitudinal wrinkles distinct, node brown. Light in weight and texture fragile. Fracture mostly hollow in centre or white pith visible. Odour slight, taste slightly acidic (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis consists of 1 layer of cells, covered with a thin layer of cuticle, mostly non-glandular hairs fallen off after staining process. Collenchyma consists of 4-5 layer of cells. Cortex parenchymatous cells irregular in shape. Phloem narrow, arranged in a ring. Mostly, xylem vessels single scattered. Pith relatively large, mostly hollow in the centre; sometimes raphides of calcium oxalate visible; polychromatic under the polarized microscope (Fig. 2).

Powder

Colour yellowish-brown. Raphides of calcium oxalate visible; polychromatic under the polarized microscope. Non-glandular hair composed of 1-20 cells, some non-glandular hairs with short branch, 13-48 μm in diameter. Fibres pit distinct. Vessels mostly spiral type (Fig. 3).

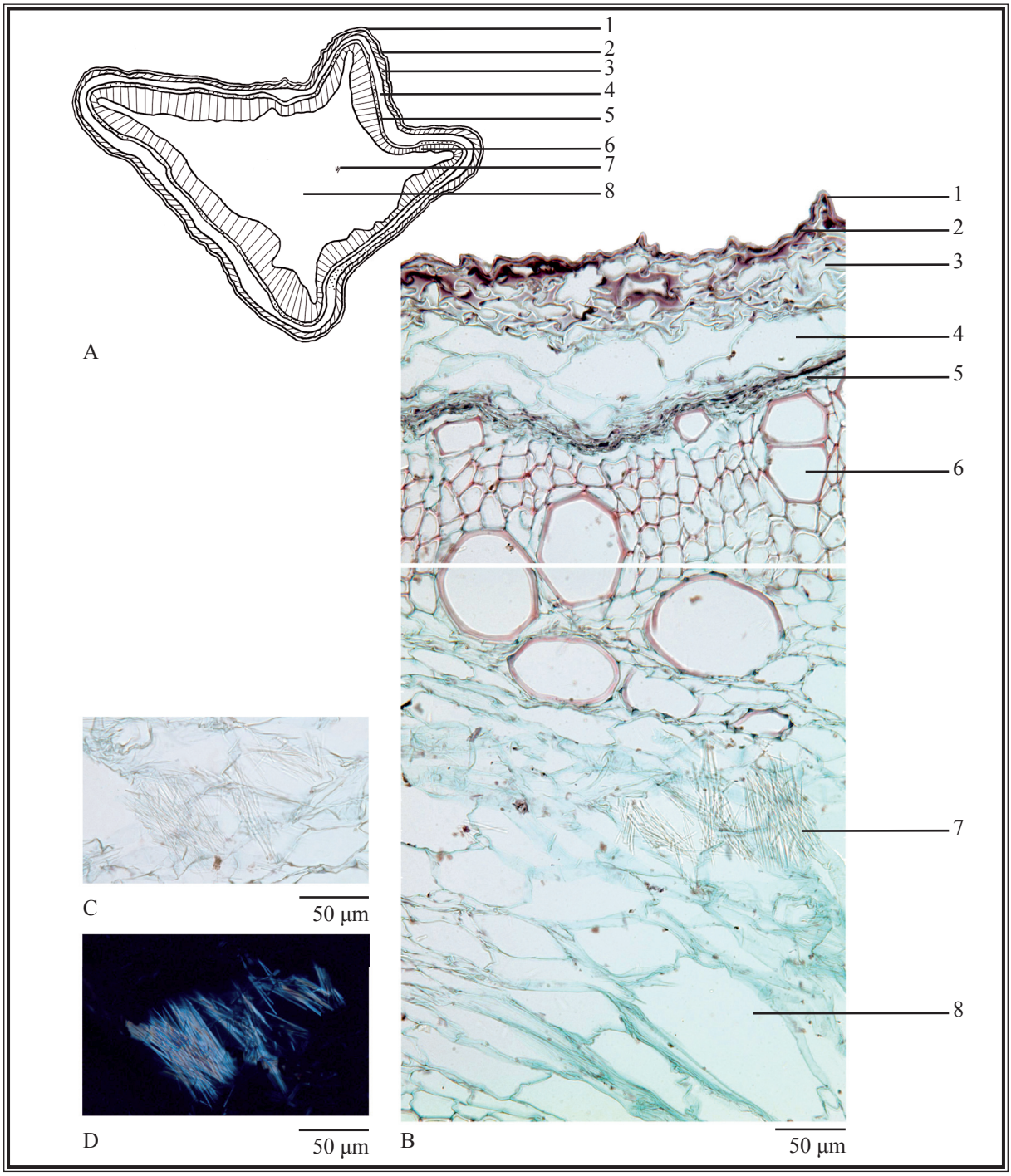


Figure 2 Microscopic features of transverse section of Impatientis Caulis

A. Sketch B. Section illustration

C. Raphides of calcium oxalate (under the light microscope)

D. Raphides of calcium oxalate (under the polarized microscope)

1. Cuticle 2. Epidermis 3. Collenchyma 4. Cortex 5. Phloem 6. Xylem

7. Raphides of calcium oxalate 8. Pith

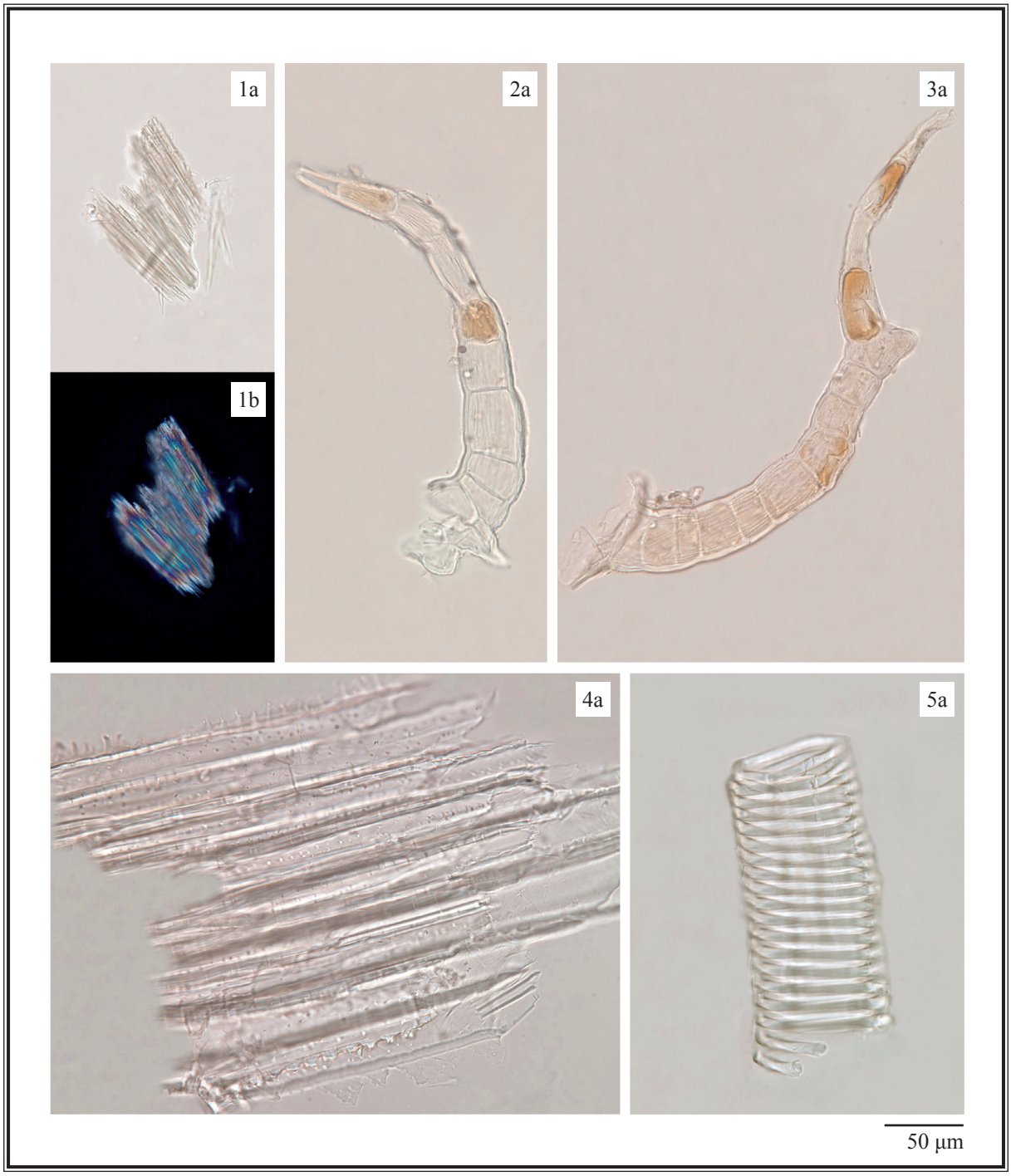


Figure 3 Microscopic features of powder of Impatientis Caulis

1. Raphides of calcium oxalate 2. Non-glandular hair
3. Non-glandular hair with short branch 4. Fibres 5. Spiral vessel

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

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

Standard solution

Scopoletin standard solution

Weigh 1.0 mg of scopoletin CRS (Fig. 4) and dissolve in 5 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60 - 80ºC), ethyl acetate and formic acid (3 : 2 : 0.1, v/v).

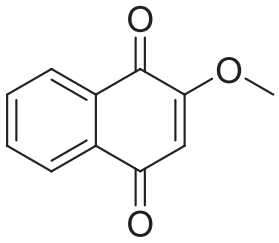
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol. Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate 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 scopoletin standard solution (1 µL) and the test solution (3 µL) to the plate. 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 (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

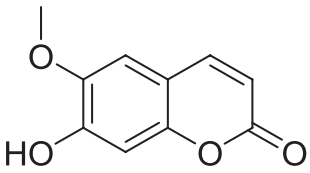


Figure 4 Chemical structures of (i) 2-methoxy-1,4-naphthoquinone and (ii) scopoletin

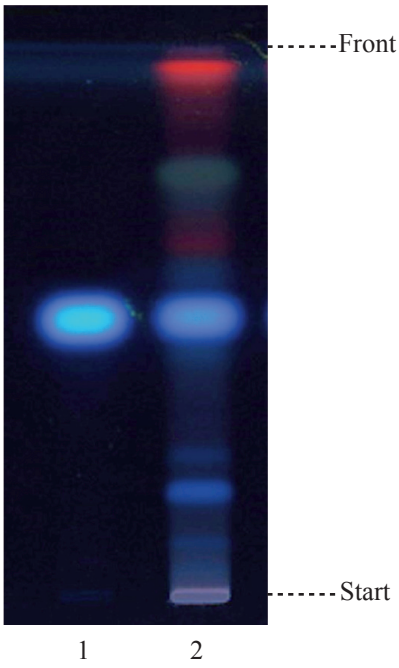


Figure 5 A reference HPTLC chromatogram of Impatientis Caulis extract observed under UV light (366 nm)

1. Scopoletin 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 scopoletin (Fig. 5).

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

Standard solutions

2-Methoxy-1,4-naphthoquinone standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 1.5 mg of 2-methoxy-1,4-naphthoquinone CRS (Fig. 4) and dissolve in 100 mL of ethanol (50%).

Scopoletin standard solution for fingerprinting, Std-FP (2 mg/L)

Weigh 0.2 mg of scopoletin CRS and dissolve in 100 mL of ethanol (50%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). 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 volumetric flask. Repeat the extraction 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 (344 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 130 Å pore size and 185 m^2/g surface area). 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 – 50	90 → 50	10 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of 2-methoxy-1,4-naphthoquinone Std-FP and scopoletin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of 2-methoxy-1,4-naphthoquinone and scopoletin should not be more than 5.0%; the RSD of the retention times of 2-methoxy-1,4-naphthoquinone and scopoletin peaks should not be more than 2.0%; the column efficiencies determined from 2-methoxy-1,4-naphthoquinone and scopoletin peaks should not be less than 40000 theoretical plates.

The *R* value between peak 1 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 2-methoxy-1,4-naphthoquinone Std-FP, scopoletin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of 2-methoxy-1,4-naphthoquinone and scopoletin peaks in the chromatograms of 2-methoxy-1,4-naphthoquinone Std-FP, scopoletin Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify 2-methoxy-1,4-naphthoquinone and scopoletin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of 2-methoxy-1,4-naphthoquinone Std-FP and scopoletin Std-FP. The retention times of 2-methoxy-1,4-naphthoquinone and scopoletin 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 Impatientis Caulis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Impatientis Caulis extract

Peak No.	RRT	Acceptable Range
1 (marker, scopoletin)	1.00	-
2	1.05	± 0.03
3	1.14	± 0.03
4	1.21	± 0.03
5 (2-methoxy-1,4-naphthoquinone)	1.81	± 0.03
6	2.19	± 0.03

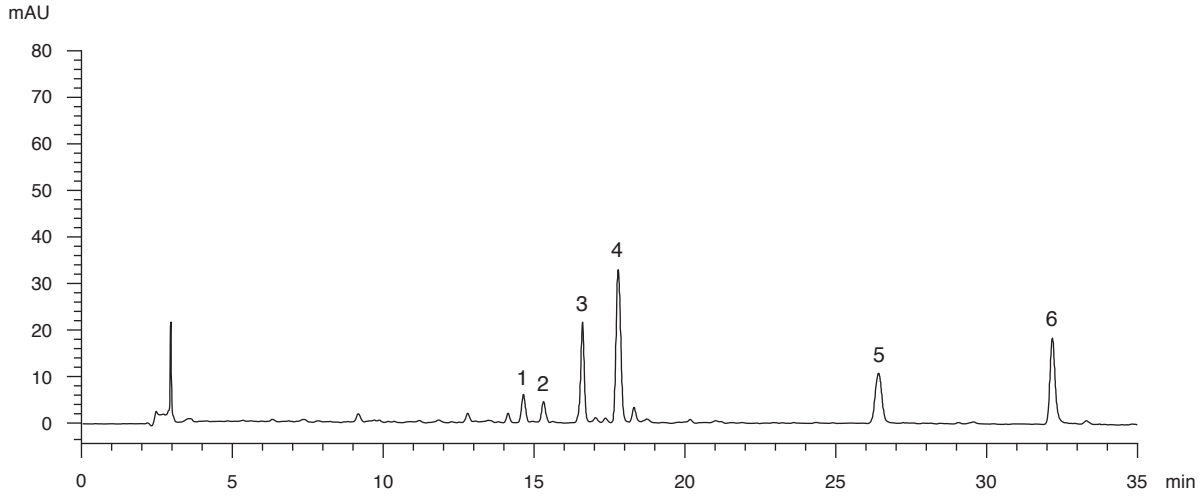


Figure 6 A reference fingerprint chromatogram of Impatientis Caulis extract

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 *(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 17.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 14.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

2-Methoxy-1,4-naphthoquinone standard stock solution, Std-Stock (50 mg/L)

Weigh accurately 0.5 mg of 2-methoxy-1,4-naphthoquinone CRS and dissolve in 10 mL of ethanol (50%).

2-Methoxy-1,4-naphthoquinone standard solution for assay, Std-AS

Measure accurately the volume of the 2-methoxy-1,4-naphthoquinone Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.5, 1, 2, 5, 50 mg/L for 2-methoxy-1,4-naphthoquinone.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). 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 volumetric flask. Repeat the extraction 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 (278 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 130 Å pore size and 185 m^2/g surface area). 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 – 50	90 → 50	10 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of 2-methoxy-1,4-naphthoquinone Std-AS (2 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of 2-methoxy-1,4-naphthoquinone should not be more than 5.0%; the RSD of the retention time of 2-methoxy-1,4-naphthoquinone peak should not be more than 2.0%; the column efficiency determined from 2-methoxy-1,4-naphthoquinone peak should not be less than 40000 theoretical plates.

The *R* value between 2-methoxy-1,4-naphthoquinone 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 2-methoxy-1,4-naphthoquinone Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of 2-methoxy-1,4-naphthoquinone against the corresponding concentrations of 2-methoxy-1,4-naphthoquinone 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 2-methoxy-1,4-naphthoquinone peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of 2-methoxy-1,4-naphthoquinone Std-AS. The retention times of 2-methoxy-1,4-naphthoquinone 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 2-methoxy-1,4-naphthoquinone in the test solution, and calculate the percentage content of 2-methoxy-1,4-naphthoquinone in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.010% of 2-methoxy-1,4-naphthoquinone ($C_{11}H_8O_3$), calculated with reference to the dried substance.

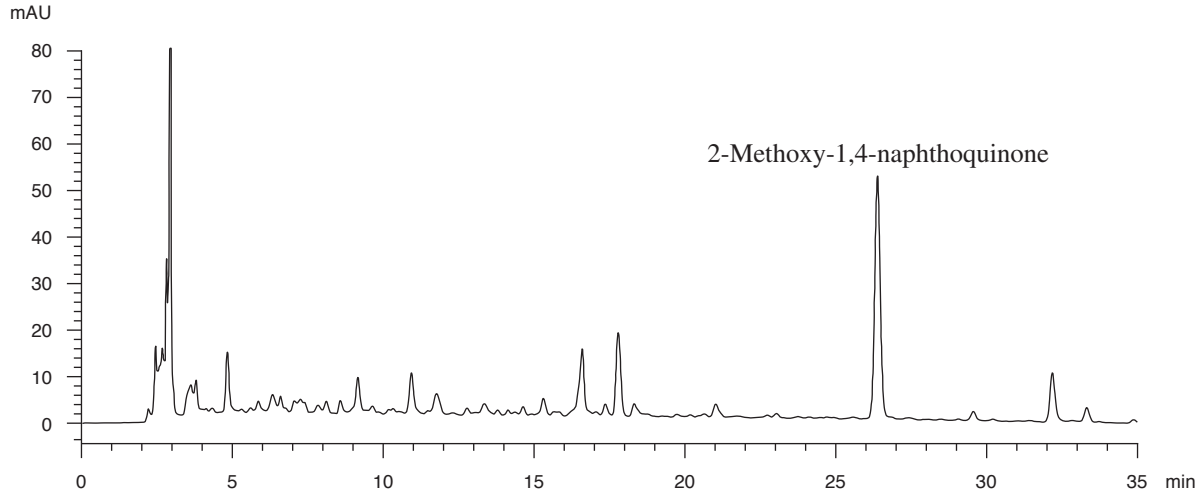


Figure 7 A reference assay chromatogram of Impatientis Caulis extract