Herba Andrographidis

Figure 1 A photograph of Herba Andrographidis

1. NAMES

Official Name: Herba Andrographidis

Chinese Name: 穿心蓮

Chinese Phonetic Name: Chuanxinlian

2. SOURCE

Herba Andrographidis is the dried aerial parts of *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae). The herb is collected in early autumn, foreign matter removed, then dried under the sun to obtain Herba Andrographidis.

3. DESCRIPTION

Stem quadrangular and frequently branched, 2-50 cm long, 2-5 mm in diameter, nodes slightly swollen. Texture fragile, easily broken. Leaves simple, opposite, petiole short or nearly sessile. Lamina crumpled and easily broken, but when wholly intact, lanceolate to ovate-lanceolate, the apex acuminate, the base cuneate-decurrent, and the margins entire to undulate; the upper surface green, the lower surface greyish-green, glabrous on both surfaces. Odour slight; taste extremely bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Epidermis consists of a layer of cutinized cells with a few glandular hairs; some cells contain a crystal of calcium carbonate (cystolith). Collenchyma dense at the four corners of the stems. Parenchyma tissue in cortex contains chloroplastids. Endodermis is composed of a layer of thicker-walled cells. Phloem narrow. Xylem well developed, composed of vessels, xylem fibres and xylem ray cells. The pith is composed of large parenchyma cells, with small acicular crystals of calcium oxalate occurring throughout.

Leaf: Upper epidermis consists of a layer of subsquare or rectangular cells, some containing a globular to elongated-ellipsoid to clavate cystolith crystal. Coniform, 1-4-celled non-glandular hairs of up to 195 μm in length, and sometimes glandular scales are also present. Palisade tissue consisting of a row of columnar cells lines up the upper part of the midrib, with collenchyma tissue beneath the epidermis. The spongy tissue consists of 4-5 rows of loosely organized parenchyma

cells, with large air spaces present. Xylem tissue is situated on the upper part, while the phloem on the lower part; they make up the collateral and grooved vascular bundles, with crystal-containing cells occurring above the xylem. Lower epidermis consists of a layer of subsquare cells, with cystoliths present (Fig. 2).

Powder

Colour green. Coniform, 1-4-celled non-glandular hairs of up to 195 μm long are present, their tip may be blunt or sharp, and with cuticular striations at base. Vessels are of spiral or reticulate type, 11-43 μm in diameter. Epidermis with glandular hairs are found. Ovoid, ellipsoid, subglobular cystolith-containing cells abundant, 36-148 μm long, 12-45 μm wide. Most of the stomata are diacytic. 4-, 6- or 8-celled glandular scales are present, 22-37 μm in diameter (Fig. 3).

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

Standard solutions

Andrographolide standard solution

Weigh 1.0 mg of andrographolide CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Dehydroandrographolide standard solution

Weigh 0.5 mg of dehydroandrographolide CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and ethanol (4:2:1, v/v).

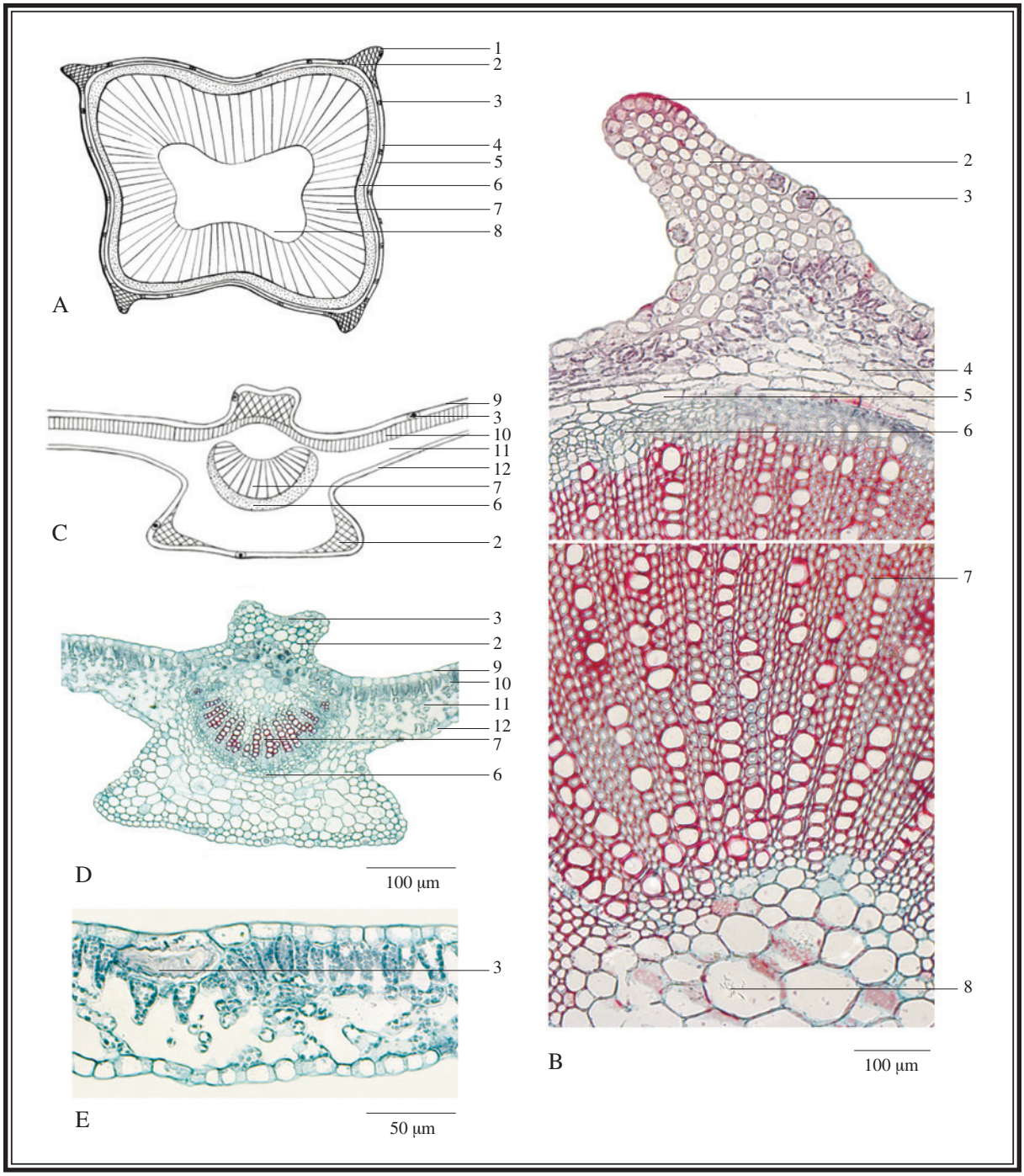


Figure 2 Microscopic features of transverse section of Herba Andrographidis

A. Sketch of stem B. Section illustration of stem

C. Sketch of leaf D. Section illustration of leaf

E. A cystolith crystal shown in leaf

1. Epidermal cells 2. Collenchyma 3. Cystolith 4. Cortex 5. Endodermis 6. Phloem

7. Xylem 8. Pith with acicular crystal of calcium oxalate 9. Upper epidermal cells 10. Palisade tissue

11. Spongy tissue 12. Lower epidermal cells

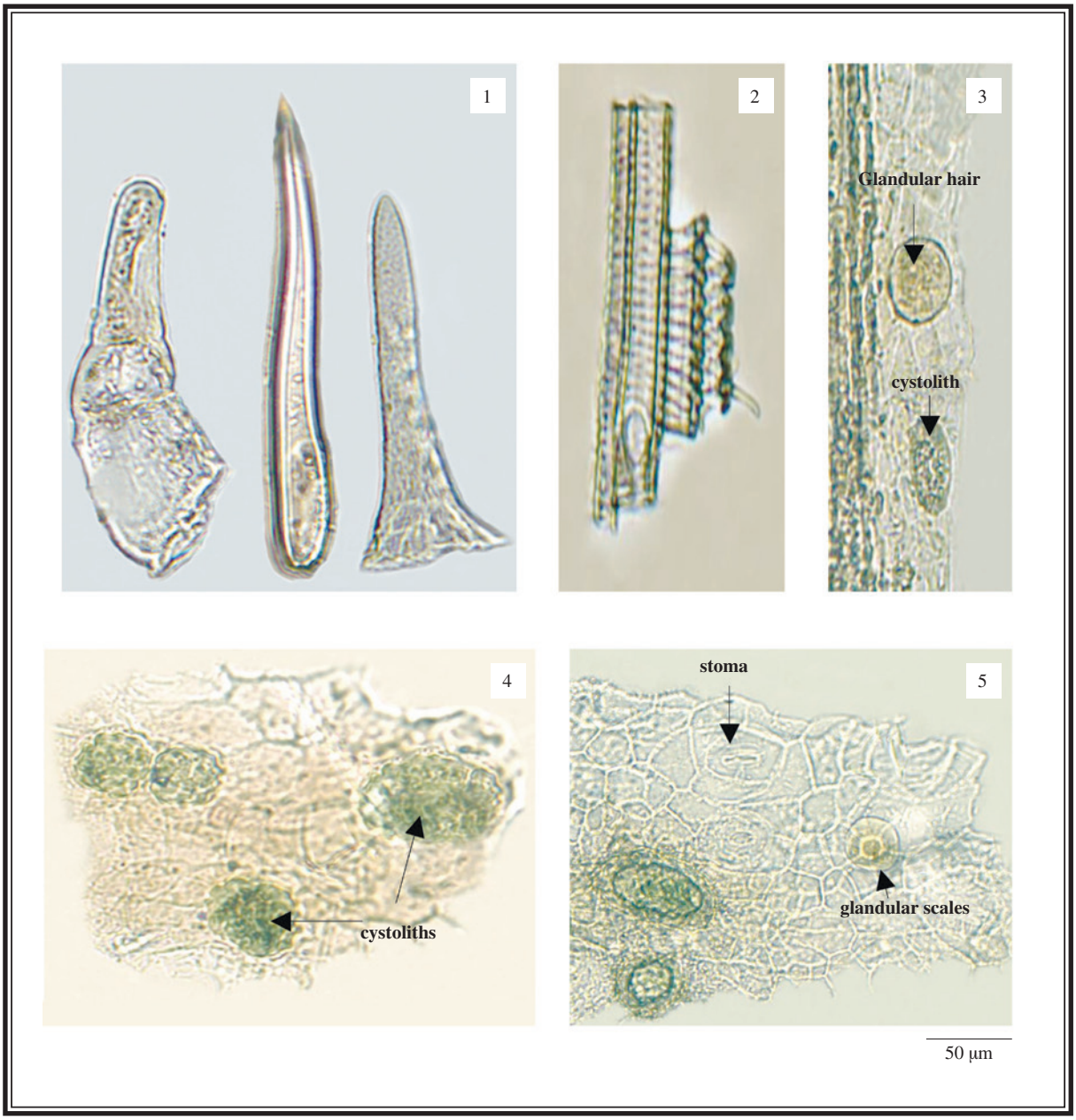


Figure 3 Microscopic features of powder of Herba Andrographidis (under the light microscope)

1. Non-glandular hairs 2. Vessels 3. Epidermis with a glandular hair and cystolith
4. Cystoliths 5. Epidermis with glandular scales and stoma (surface view)

Test solution

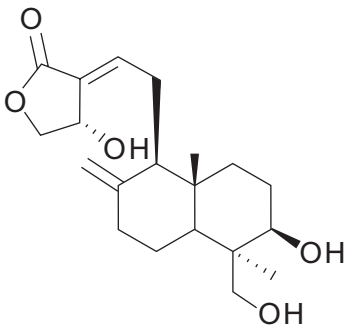
Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of ethanol. Sonicate (240 W) the mixture for 20 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol.

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 andrographolide standard solution (5 μL) and dehydroandrographolide standard solution (6 μL) and the test solution (3 μL) 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 7.5 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).

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 andrographolide and dehydroandrographolide.

(i)



(ii)

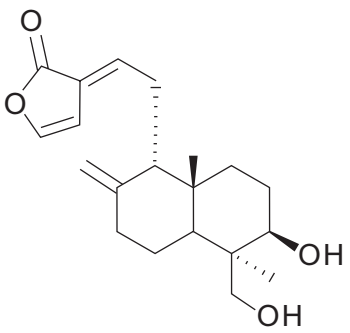


Figure 4 Chemical structures of (i) andrographolide and (ii) dehydroandrographolide

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

Standard solutions

Andrographolide standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 3.0 mg of andrographolide CRS and dissolve in 50 mL of methanol.

Dehydroandrographolide standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 1.5 mg of dehydroandrographolide CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatant. Make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 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
0–5	78→75	22→25	linear gradient
5–30	75→70	25→30	linear gradient
30–60	70→55	30→45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of andrographolide Std-FP and dehydroandrographolide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of andrographolide and dehydroandrographolide should not be more than 5.0%; the RSD of the retention times of andrographolide and dehydroandrographolide peaks should not be more than 2.0%; the column efficiencies determined from andrographolide and dehydroandrographolide peaks should not be less than 8000 and 40000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig.5).

Procedure

Separately inject andrographolide Std-FP, dehydroandrographolide Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of andrographolide and dehydroandrographolide peaks in the chromatograms of the corresponding Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify andrographolide and dehydroandrographolide peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatograms of the corresponding Std-FP. The retention times of andrographolide and dehydroandrographolide 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 four characteristic peaks of Herba Andrographidis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Herba Andrographidis extract

Peak No.	RRT	Acceptable Range
1 (marker 1, andrographolide)	1.00	-
2	0.85 (vs peak 4)	±0.03
3	0.97 (vs peak 4)	±0.03
4 (marker 2, dehydroandrographolide)	1.00	-

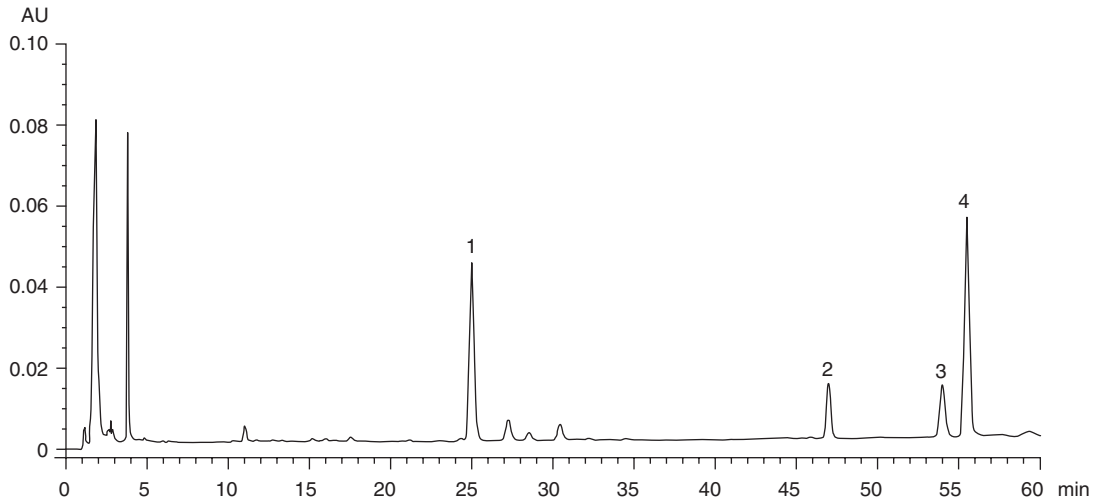


Figure 5 A reference fingerprint chromatogram of Herba Andrographidis extract

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 XV)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 13.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*: not more than 11.0%.

5.8 Leaves: not less than 30%.

Note: *The leaves ratio refers to the weight of leaves against the total weight of the CMM sample.*

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 16.0%.
Ethanol-soluble extractives (hot extraction method): not less than 15.0%.

7. ASSAY

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

Standard solution

Mixed andrographolide and dehydroandrographolide standard stock solution, Std-Stock (200 mg/L for andrographolide and 100 mg/L for dehydroandrographolide)

Weigh accurately 5.0 mg of andrographolide CRS and 2.5 mg of dehydroandrographolide CRS and dissolve in 25 mL of methanol.

Mixed andrographolide and dehydroandrographolide standard solution for assay, Std-AS

Measure accurately the volume of the mixed andrographolide and dehydroandrographolide Std-Stock, dilute with methanol to produce a series of solutions of 20, 40, 60, 100, 150 mg/L for andrographolide and 10, 20, 30, 50, 75 mg/L for dehydroandrographolide.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatant. Make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (225 nm for andrographolide and 254 nm for dehydroandrographolide) 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 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–5	78→75	22→25	linear gradient
5–30	75→70	25→30	linear gradient
30–60	70→55	30→45	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed andrographolide and dehydroandrographolide Std-AS (60 mg/L for andrographolide and 30 mg/L for dehydroandrographolide). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of andrographolide and dehydroandrographolide should not be more than 5.0%; the RSD of the retention times of andrographolide and dehydroandrographolide peaks should not be more than 2.0%; the column efficiencies determined from andrographolide and dehydroandrographolide peaks should not be less than 8000 and 40000 theoretical plates respectively.

The *R* value between andrographolide peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 and the *R* value between dehydroandrographolide 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 andrographolide and dehydroandrographolide Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of andrographolide and dehydroandrographolide against the corresponding concentrations of the mixed andrographolide and dehydroandrographolide 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 andrographolide peak and dehydroandrographolide peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed andrographolide and dehydroandrographolide Std-AS. The retention times of andrographolide peaks and dehydroandrographolide peaks from the two chromatograms should not differ from their counterparts by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of andrographolide and dehydroandrographolide in the test solution, and calculate the percentage contents of andrographolide and dehydroandrographolide in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.80% of the total content of andrographolide ($C_{20}H_{30}O_5$) and dehydroandrographolide ($C_{20}H_{28}O_4$), calculated with reference to the dried substance.