Dipsaci Radix

Figure 1 A photograph of Dipsaci Radix

A. Dipsaci Radix B. Transverse section

1. NAMES

Official Name: Dipsaci Radix

Chinese Name: 續斷

Chinese Phonetic Name: Xuduan

2. SOURCE

Dipsaci Radix is the dried root of *Dipsacus asper* Wall. ex Henry (Dipsacaceae). The root is collected in autumn, root stock and rootlets removed, piled up at room temperature until a green colour is developed inside, and then baked at a temperature about 45-60ºC until dryness to obtain Dipsaci Radix.

3. DESCRIPTION

Cylindrical, somewhat flattened, slightly curved, 5-20 cm long, 4-20 mm in diameter. Externally greyish-brown or yellowish-brown, with slightly twisted or obviously twisted longitudinal wrinkles and furrows, showing transversal lenticels-like cicatrices and sparse rootlet scars. Texture soft but hardened after a long period of storage, easily broken. Fracture uneven, bark dark green to brown, the outer part pale brown to brown, wood yellowish-brown to greenish-brown, vessel bundles arranged radially. Odour slightly aromatic; taste bitter, with slight sweetish and followed by astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells. Phelloderm narrow, consists of 6-8 layers of cells. Groups of sieve tubes sparsely scattered in the phloem. Parenchymatous cells contain clusters of calcium oxalate. Cambium distinct or less distinct. Xylem ray broad; vessels dense near the cambium and lessened inward. Pith small, and most absent in small roots (Fig. 2).

Powder

Colour yellowish-brown. Clusters of calcium oxalate fairly abundant, scattered or embedded in shrunken parenchymatous cells, 15-50 μm in diameter, sometimes arranged as a strip; polychromatic under the polarized microscope. Some parenchymatous cells with fine, oblique crisscross striations, wall slightly thickened. Vessels bordered-pitted and reticulate, varying in diameter (occasionally up to 70 μm). Cork cells pale brown, subrectangular, subsquare, polygonal or elongated-polygonal in surface view, wall thin (Fig. 3).

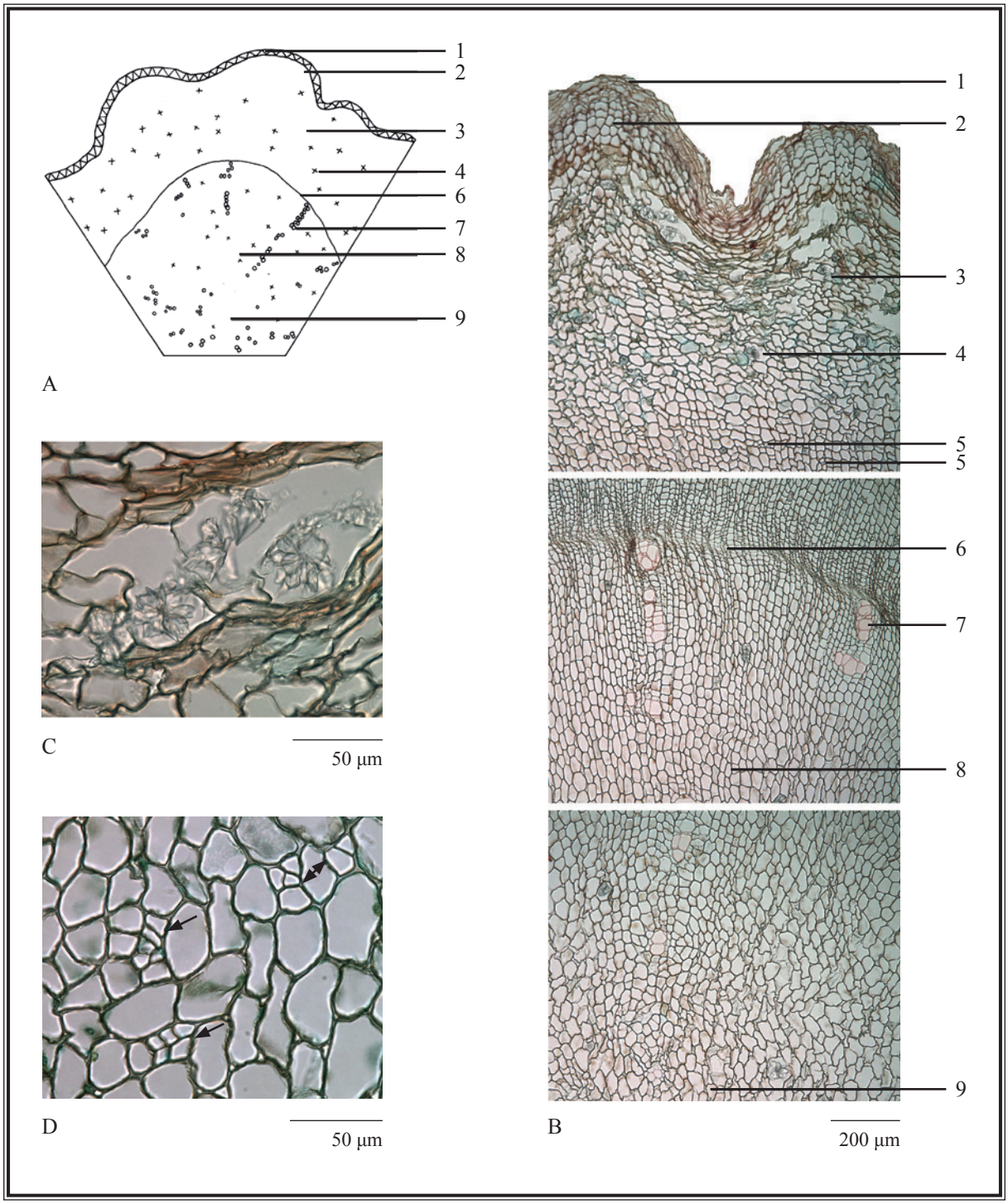

Figure 2 Microscopic features of transverse section of Dipsaci Radix

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

1. Cork 2. Phelloderm 3. Phloem 4. Clusters of calcium oxalate
5. Sieve tubes 6. Cambium 7 . Xylem 8. Xylem ray 9. Pith

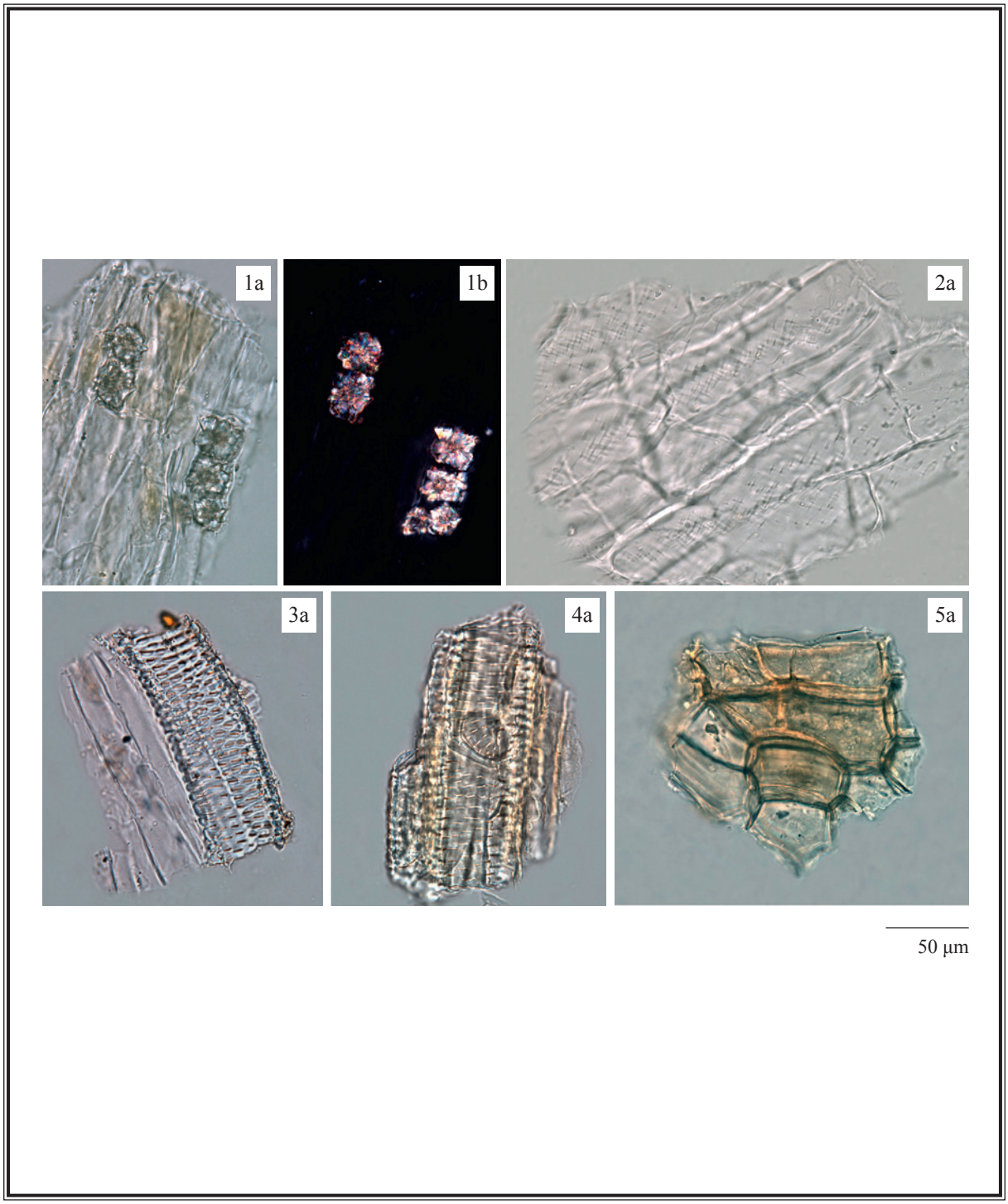

Figure 3 Microscopic features of powder of Dipsaci Radix

1. Clusters of calcium oxalate 2. Parenchymatous cells with oblique crisscross striations
3. Bordered-pitted vessel 4. Reticulate vessels 5. Cork cells

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

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

Standard solution

Asperosaponin VI standard solution

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

Developing solvent system

Prepare a mixture of water, n-butanol and acetic acid (5:4:1, v/v). Use the upper layer.

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL conical flask, then add 8 mL of ethanol. 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 asperosaponin VI standard solution (2 µL) and the test solution (3 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. Spray the plate evenly with the spray reagent and heat at about 105ºC until the spots or bands become visible (about 5-10 min). 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 asperosaponin VI.

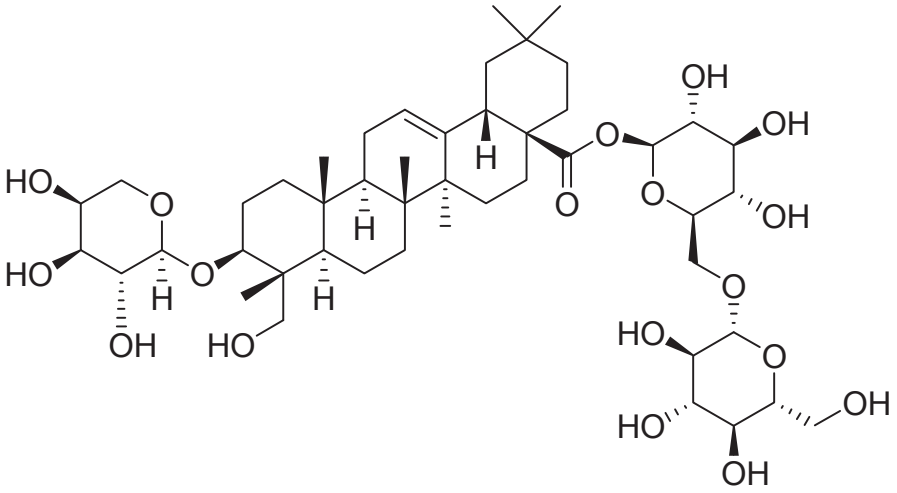

Figure 4 Chemical structure of asperosaponin VI

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

Standard solution

Asperosaponin VI standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 5.0 mg of asperosaponin VI CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. 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 methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 110ºC; nebulizer gas (N_2) flow: 2.0 L/min] 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	90 → 55	10 → 45	linear gradient
40 – 60	55 → 5	45 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of asperosaponin VI Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of asperosaponin VI should not be more than 5.0%; the RSD of the retention time of asperosaponin VI peak should not be more than 2.0%; the column efficiency determined from asperosaponin VI peak should not be less than 250000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

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

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Dipsaci Radix extract

Peak No.	RRT	Acceptable Range
1	0.69	± 0.03
2	0.74	± 0.03
3	0.90	± 0.03
4 (marker, asperosaponin VI)	1.00	-

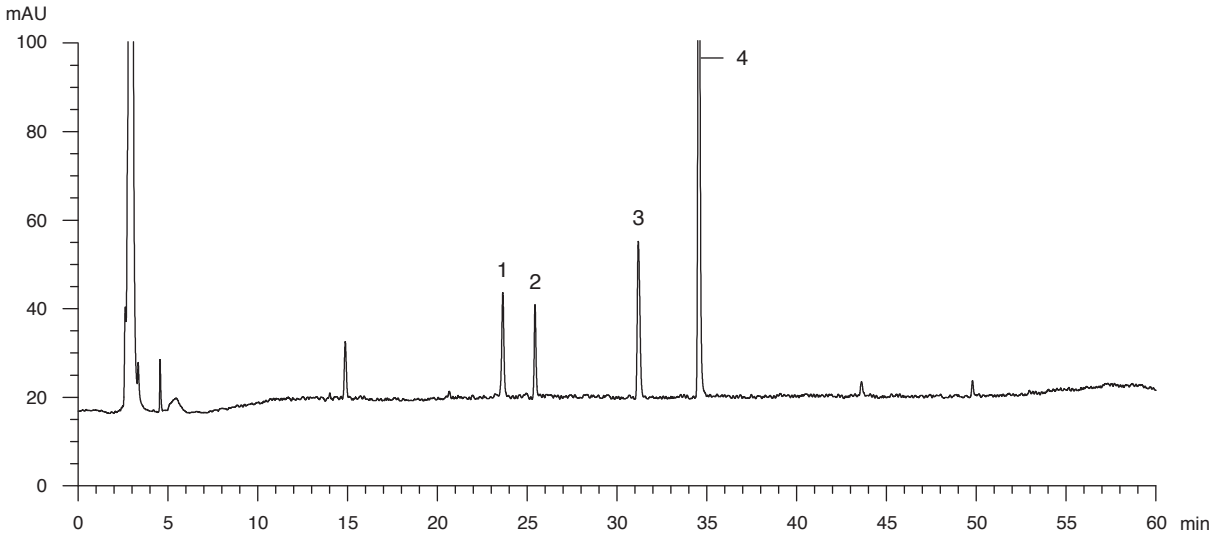

Figure 5 A reference fingerprint chromatogram of Dipsaci Radix extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.0%.
Acid-insoluble ash: not more than 3.0%.

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 41.0%.
Ethanol-soluble extractives (cold extraction method): not less than 36.0%.

7. ASSAY

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

Standard solution

Asperosaponin VI standard stock solution, Std-Stock (3000 mg/L)

Weigh accurately 3.0 mg of asperosaponin VI CRS and dissolve in 1 mL of methanol.

Asperosaponin VI standard solution for assay, Std-AS

Measure accurately the volume of the asperosaponin VI Std-Stock, dilute with methanol to produce a series of solutions of 100, 200, 400, 600, 1000 mg/L for asperosaponin VI.

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 methanol. 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 methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 110ºC; nebulizer gas (N_2) flow: 2.0 L/min] 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 – 40	90 → 55	10 → 45	linear gradient
40 – 60	55 → 5	45 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of asperosaponin VI Std-AS (400 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of asperosaponin VI should not be more than 5.0%; the RSD of the retention time of asperosaponin VI peak should not be more than 2.0%; the column efficiency determined from asperosaponin VI peak should not be less than 250000 theoretical plates.

The *R* value between asperosaponin VI 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 asperosaponin VI Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of asperosaponin VI against the natural logarithm of the corresponding concentrations of asperosaponin VI 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 asperosaponin VI peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of asperosaponin VI Std-AS. The retention times of asperosaponin VI 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 asperosaponin VI in the test solution by using the following equation –

$$\text{Concentration of asperosaponin VI in the test solution} = e^{[\text{Ln(A)}-I]/m}$$

Where A = the peak area of asperosaponin VI in the test solution,

I = the y-intercept of the 5-point calibration curve of asperosaponin VI,

m = the slope of the 5-point calibration curve of asperosaponin VI.

Calculate the percentage content of asperosaponin VI in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 2.0% of asperosaponin VI ($C_{47}H_{76}O_{18}$), calculated with reference to the dried substance.