Viticis Negundo Folium

Figure 1 A photograph of Viticis Negundo Folium

A. Viticis Negundo Folium

B. Upper surface of palmately compound leaf

C. Lower surface of palmately compound leaf

1. NAMES

Official Name: Viticis Negundo Folium

Chinese Name: 牡荊葉

Chinese Phonetic Name: Mujingye

2. SOURCE

Viticis Negundo Folium is the dried leaf of *Vitex negundo* L. var. *cannabifolia* (Sieb. et Zucc.) Hand.-Mazz. (Verbenaceae). The leaf is collected in summer and autumn when foliage growing luxuriantly, then dried under the sun to obtain Viticis Negundo Folium.

3. DESCRIPTION

Leaves palmately compound. Leaflets 3-5, lanceolate or elliptical-lanceolate, the central leaflet larger, 2-11 cm long, 1-4 cm wide, the lateral leaflets relatively small, apex acuminate, base cuneate, margins dentate. Upper surface blackish-green, lower surface pale green, both surfaces pubescent along veins, pubescence relatively denser on the lower surface of young leaves. Petioles 1-9 cm long, with a shallow groove, densely covered with greyish-white pubescence. Odour aromatic; taste pungent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermis cells arranged regularly. Collenchyma consists of several layers of cells, located underneath the upper and lower epidermis. Palisade tissue consists of 3-4 layers of cells. Spongy tissue consists of loosely arranged cells. Vascular bundles 1-5, collateral, the primary bundle crescent or U-shaped. The small vascular bundles, scattering inside the cleft of the U-shaped vascular bundles. Non-glandular hairs raised from upper and lower epidermis, relative abundantly on the lower epidermis (Fig. 2).

Powder

Colour blackish-green to pale green. Non-glandular hairs consists of 1-4 uniseriate cells with the apical cell relatively long, with warty protuberance on the surface. Glandular scales consists of 4-celled head and unicellular stalk, 31-54 μm in diameter. Small glandular hairs consists of 1- to 4-celled head, stalk 1- to 3-celled, and very short, 15-34 μm in diameter. Lower epidermal cells subpolygonal or irregular in shape, anticlinal walls undulantly curve, with numerous infinitive stomata. Stone cells occasionally found, square, rectangular or triangular, walls thick, with clear pits and pit canals, 12-82 μm in diameter. Vessels mainly spiral (Fig. 3).

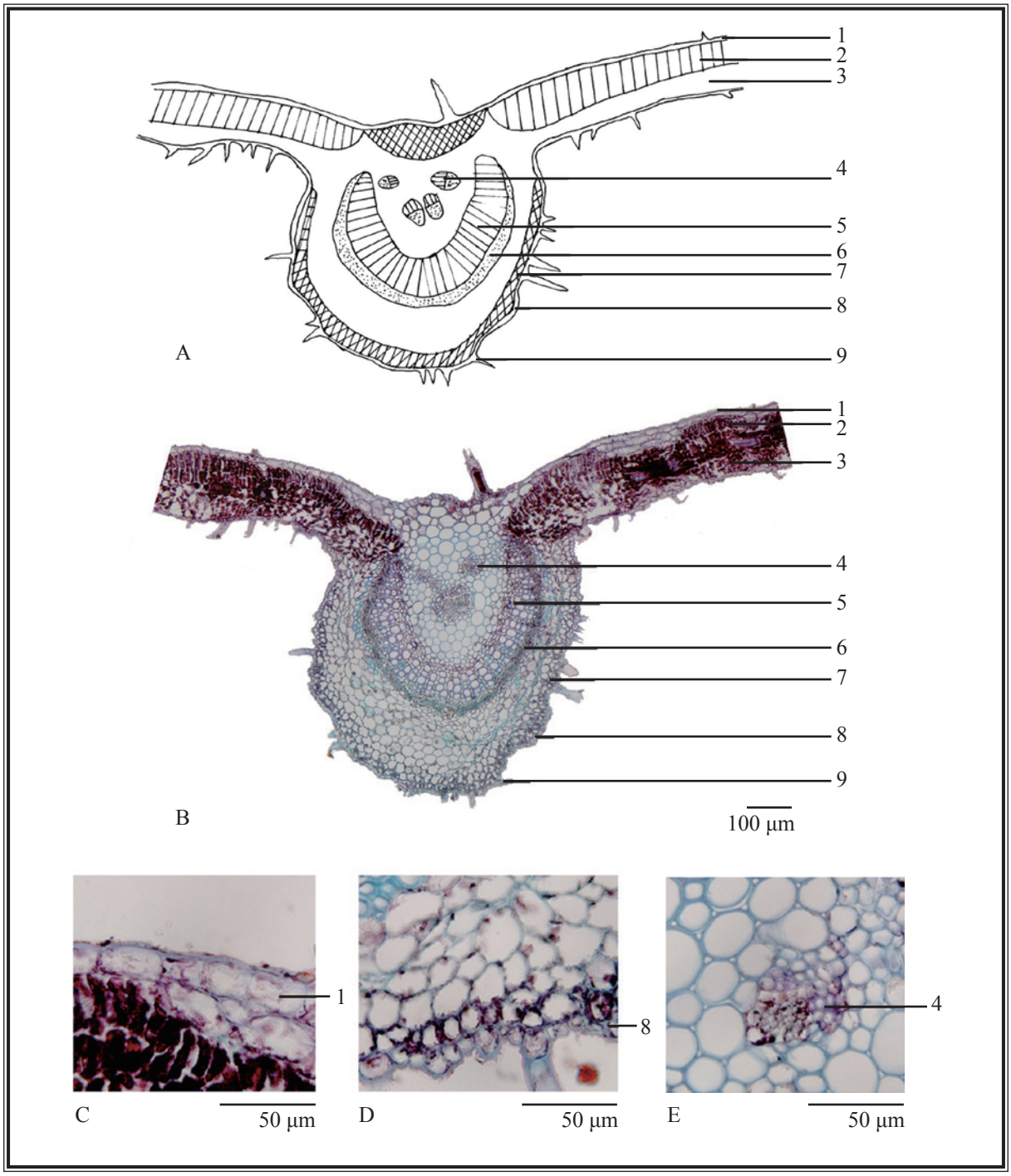

Figure 2 Microscopic features of transverse section of Viticis Negundo Folium

A. Sketch B. Section illustration C. Upper epidermis
D. Lower epidermis E. Small vascular bundle

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Small vascular bundle
5. Xylem 6. Phloem 7. Collenchyma 8. Lower epidermis 9. Non-glandular hair

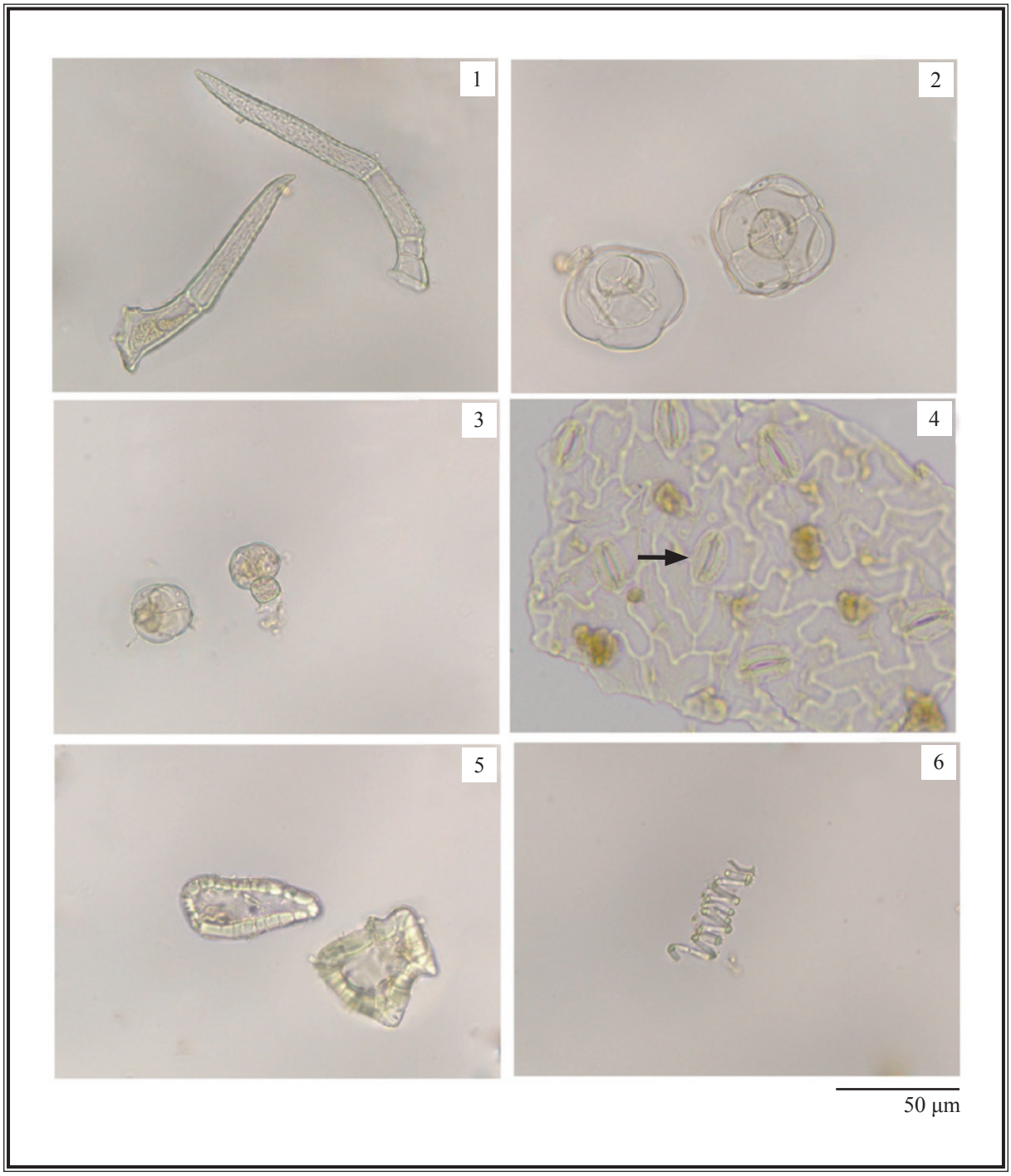

Figure 3 Microscopic features of powder of Viticis Negundo Folium (under the light microscope)

1. Non-glandular hairs 2. Glandular scales 3. Small glandular hairs
4. Lower epidermal cell with stomata (→) 5. Stone cells 6. Spiral vessel

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

Standard solution

Isovitexin standard solution

Weigh 1.0 mg of isovitexin CRS (Fig. 4) and dissolve in 20 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid and water (7.5:1:0.5, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a TLC polyamide plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately isovitexin standard solution (1.5 μL) and the test solution (2 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC plate for development. Develop over a path of about 8 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 (about 5 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

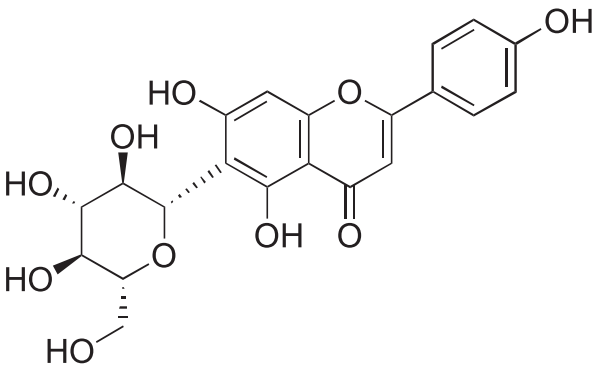

Figure 4 Chemical structure of isovitexin

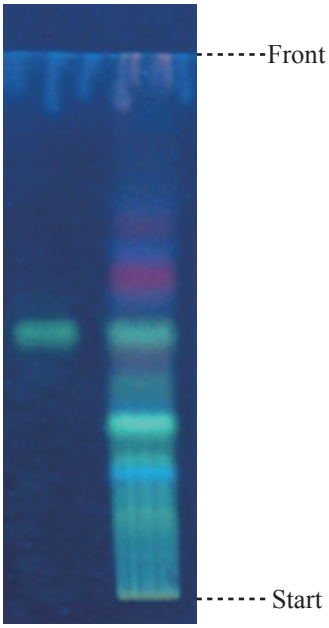

Figure 5 A reference TLC chromatogram of Viticis Negundo Folium extract observed under UV light (366 nm) after staining

1. Isovitexin 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 isovitexin (Fig. 5).

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

Standard solution

Isovitexin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 0.4 mg of isovitexin CRS and dissolve in 10 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 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)	Acetonitrile (%, v/v)	0.05% Formic acid (%, v/v)	Elution
0 – 20	15 → 20	85 → 80	linear gradient
20 – 43	20 → 23	80 → 77	linear gradient
43 – 60	23 → 40	77 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of isovitexin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isovitexin should not be more than 5.0%; the RSD of the retention time of isovitexin peak should not be more than 2.0%; the column efficiency determined from isovitexin peak should not be less than 30000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject isovitexin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention time of isovitexin peak in the chromatogram of isovitexin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify isovitexin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isovitexin Std-FP. The retention times of isovitexin 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 five characteristic peaks of Viticis Negundo Folium extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Viticis Negundo Folium extract

Peak No.	RRT	Acceptable Range
1	0.69	± 0.03
2 (marker, isovitexin)	1.00	-
3	1.10	± 0.03
4	1.80	± 0.05
5	2.46	± 0.05

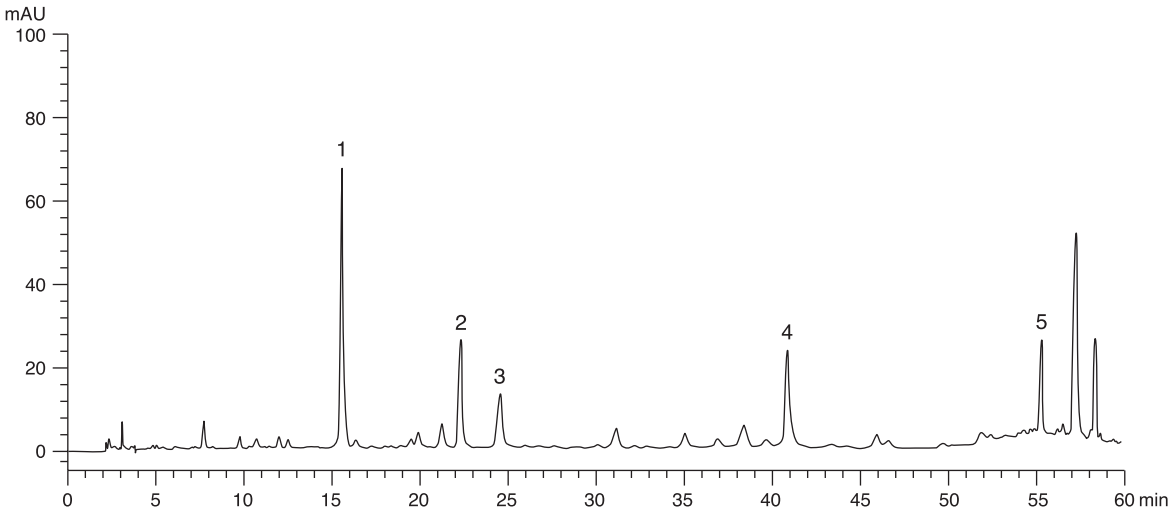

Figure 6 A reference fingerprint chromatogram of Viticis Negundo Folium extract

For positive identification, the sample must give the above five 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 3.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 7.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

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

Standard solution
Isovitexin standard stock solution, Std-Stock (500 mg/L)
Weigh accurately 5.0 mg of isovitexin CRS and dissolve in 10 mL of ethanol (70%).
Isovitexin standard solution for assay, Std-AS
Measure accurately the volume of the isovitexin Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 0.5, 5, 10, 20, 30 mg/L for isovitexin.

Test solution
Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (70%). Sonicate (150 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (70%). Combine the solutions and make up to the mark with ethanol (70%). Filter through a 0.45-µm PTFE filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (340 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. The mobile phase is a mixture of 0.05% formic acid and acetonitrile (82.5:17.5, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 5 µL of isovitexin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of isovitexin should not be more than 5.0%; the RSD of the retention time of isovitexin peak should not be more than 2.0%; the column efficiency determined from isovitexin peak should not be less than 8000 theoretical plates.

The *R* value between isovitexin 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 isovitexin Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of isovitexin against the corresponding concentrations of isovitexin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify isovitexin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isovitexin Std-AS. The retention times of isovitexin 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 isovitexin in the test solution, and calculate the percentage content of isovitexin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.048% of isovitexin ($C_{21}H_{20}O_{10}$), calculated with reference to the dried substance.