Radix Angelicae Pubescentis

Figure 1 A photograph of Radix Angelicae Pubescentis

1. NAMES

Official Name: Radix Angelicae Pubescentis

Chinese Name: 獨活

Chinese Phonetic Name: Duhuo

2. SOURCE

Radix Angelicae Pubescentis is the dried root of *Angelica pubescens* Maxim. f. *biserrata* Shan et Yuan (Apiaceae/Umbelliferae). The root is collected in early spring before sprouting or in late autumn when stem and leaves wither. After removal of stem remnant, rootlets and soil, the root is baked to half dry, piled for 2-3 days to soften, then baked to dryness to obtain Radix Angelicae Pubescentis.

3. DESCRIPTION

Somewhat cylindrical, with 2-3 branches or more at the lower part, 4.5-23 cm long. The upper part bulgy, marked with transverse wrinkles, 6-45 mm in diameter, the apex exhibiting remains of stems and leaves or sunken spots. Outer surface greyish-brown or dark brown, marked with longitudinal wrinkles, protuberant elongate lenticels and slightly protuberant rootlet scars. Texture relatively hard, softening when moistened; fracture showing bark greyish-white, scattered with numerous brown oil dots; cambium ring brown; wood greyish-yellow to yellowish-brown. Odour with distinctive fragrance; taste bitter-acrid, slightly tongue-numbing (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consisting of 3-12 layers of cells. Cortex narrow, with a few oil ducts. Phloem broad, occupying about 1/2 the radius of root, often with clefts; oil ducts relatively abundant, arranged in several rings, subround or elliptical, surrounded by 6-22 secretory cells, smaller near the cambium. Cambium in a ring. Rays 1-4 rows of cells wide. Vessels singly or in group, arranged radially in the xylem. The parenchyma cells contain starch granules (Fig. 2).

Powder

Colour pale brown or brown. Oil ducts mostly broken, in transverse view the secretory cells appear elongated-subround, 5-30 µm in diameter, mostly containing yellowish-green or pale yellowish-brown secretions and oily drops. Phloem parenchyma cells colourless or pale yellowish, spindle-shaped, wall slightly thickened, with very fine oblique crisscross striations, 7-38 µm in diameter, thin and sinuous, transverse septa visible. Starch granules abundant, small; simple granules subround or elliptical, the hilum sometimes visible in large ones, punctate or herringbone-shaped, 2-13 µm in diameter; compound granules frequent, consisting of 2 to about 10 units, somewhat discrete. Vessels mainly reticulated or spiral, 5-90 µm in diameter. Cork cells yellowish-brown, polygonal or elongated-polygonal in surface view (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 0.5 g of the powdered sample and put into a 50-mL conical flask, then add 10 mL of ethanol (95%). Allow to stand for 15 min. Filter and transfer the filtrate to another conical flask. Concentrate the filtrate to about 1 mL on a water bath at 50°C, then transfer the solution to a test tube. Add hydroxylamine hydrochloride solution (7%, w/v) and potassium hydroxide solution (20%, w/v), 3 drops each, to the test tube. Mix well and heat the mixture on a water bath at 50°C for about 10 min. Cool to room temperature. Adjust the pH to about 3–4 with dilute hydrochloric acid (10%, v/v). Add 2 drops of iron(III) chloride solution (1%, w/v) and mix well. A light brown to orange red solution is observed.

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

Standard solutions

Columbianetin acetate standard solution

Weigh 2.0 mg of columbianetin acetate CRS (Fig. 4) and dissolve in 2 mL of dichloromethane.

Osthole standard solution

Weigh 2.0 mg of osthole CRS (Fig. 4) and dissolve in 2 mL of dichloromethane.

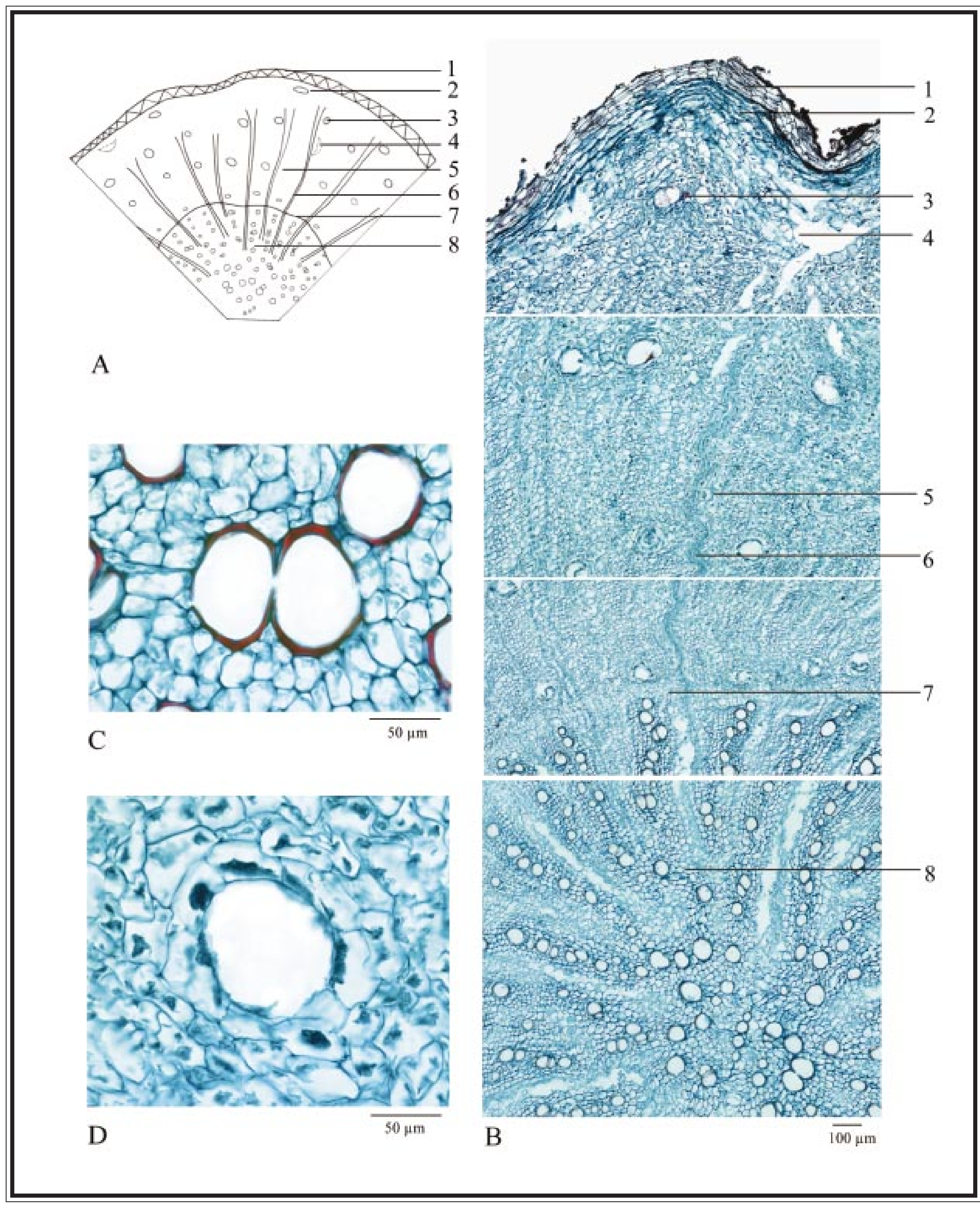


Figure 2 Microscopic features of transverse section of Radix Angelicae Pubescentis

A. Sketch B. Section illustration C. Vessels D. Oil ducts

1. Cork 2. Phelloderm 3. Oil duct 4. Clefts 5. Phloem 6. Ray 7. Cambium 8. Xylem

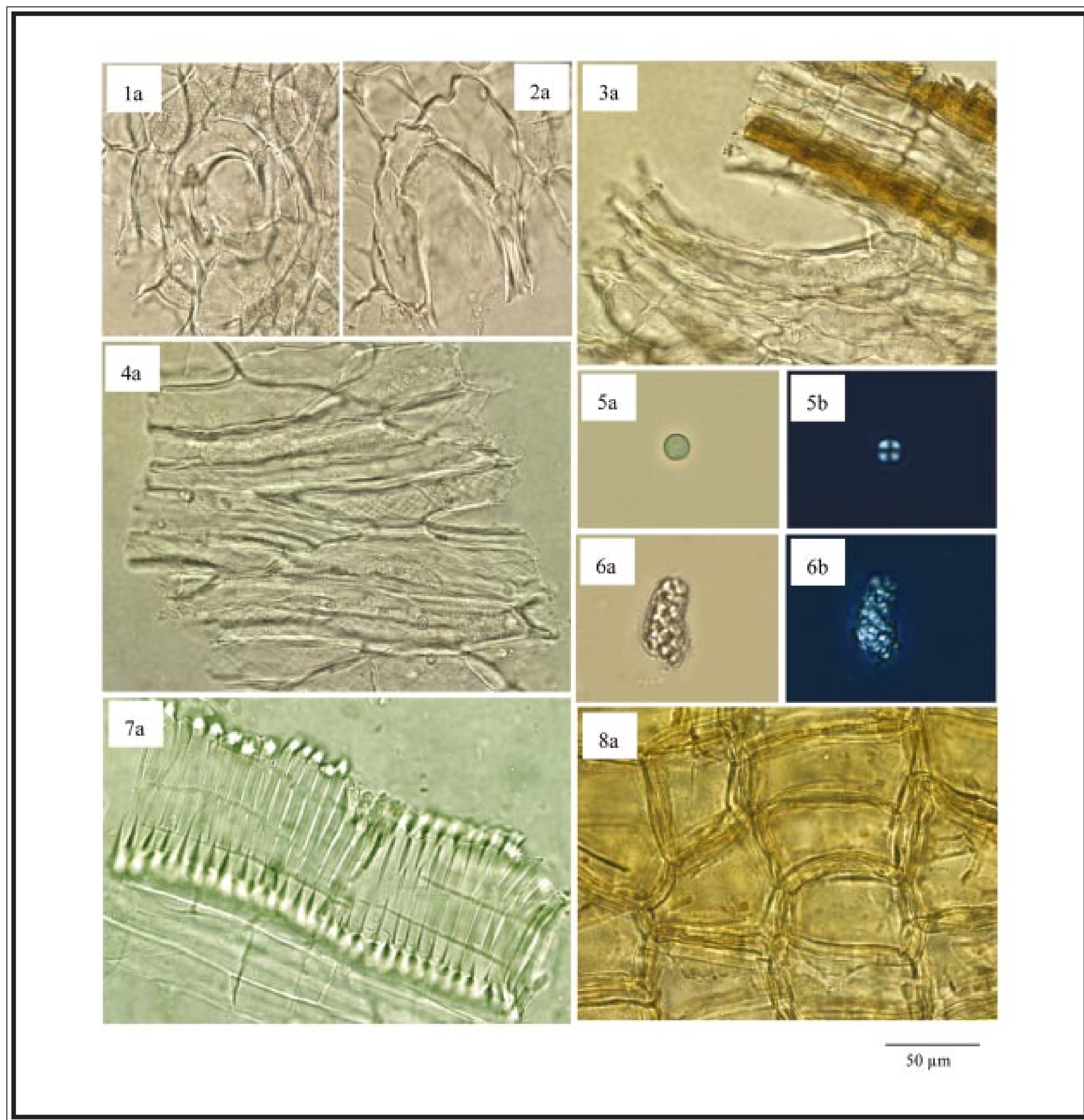


Figure 3 Microscopic features of powder of Radix Angelicae Pubescentis

1. Oil duct   2. Fragments of oil duct   3. Oil duct with yellowish-brown secretions
4. Parenchyma cells with crisscross striations   5. Simple starch granule   6. Compound starch granules
7. Reticulated vessel   8. Cork cells

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

Developing solvent system

Prepare a mixture of *n*-hexane, toluene and ethyl acetate (2:1:1, v/v).

Test solution

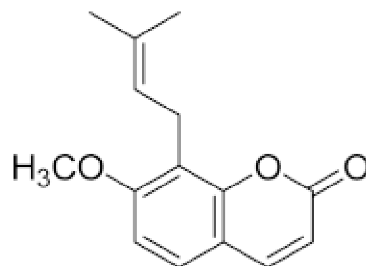
Weigh 2.0 g of the powdered sample and put into a 50-mL conical flask, then add 10 mL of diethyl ether. Allow to stand overnight. Filter and transfer the filtrate to another conical flask. Evaporate to dryness on a water bath at 35°C. Dissolve the residue in 2 mL of dichloromethane.

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 columbianetin acetate standard solution, osthole standard solution and the test solution (2 µL each) to the plate. Develop over a path of about 9 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 and 365 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 columbianetin acetate and osthole.

(i)



(ii)

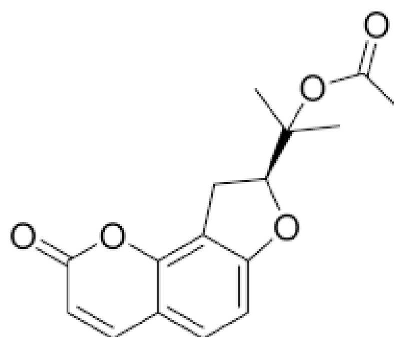


Figure 4 Chemical structures of (i) osthole and (ii) columbianetin acetate

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

Standard solution

Osthole standard stock solution, Std-Stock (44 mg/L)

Weigh 2.2 mg of osthole CRS and dissolve in 50 mL of methanol.

Osthole standard solution for fingerprinting, Std-FP (8.8 mg/L)

Pipette 0.4 mL of osthole Std-Stock into a 2-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 0.1 g of the powdered sample and put into a 250-mL conical flask, then add 100 mL of methanol. Sonicate (220 W) the mixture for 10 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (320 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 –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 15	70	30	isocratic
15 – 20	70 → 55	30 → 45	linear gradient
20 – 40	55 → 35	45 → 65	linear gradient

System suitability requirements

Perform at least five replicate injections each with 20 µL of osthole Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of osthole should not be more than 3.0%; the RSD of the retention time of osthole peak should not be more than 2.0%; the column efficiency determined from osthole peak should not be less than 10000 theoretical plates.

The *R* value between peak 2 and peak 3 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject osthole Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention time of osthole peak in the chromatogram of osthole Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram

of the test solution. Under the same HPLC conditions, identify osthole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of osthole Std-FP. The retention times of osthole 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 six characteristic peaks of Radix Angelicae Pubescentis extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the six characteristic peaks of Radix Angelicae Pubescentis extract

Peak No.	RRT	Acceptable Range
1	0.51	± 0.03
2	0.61	± 0.03
3	0.63	± 0.03
4 (columbianetin acetate)	0.75	± 0.03
5 (marker, osthole)	1.00	-
6	1.08	± 0.03

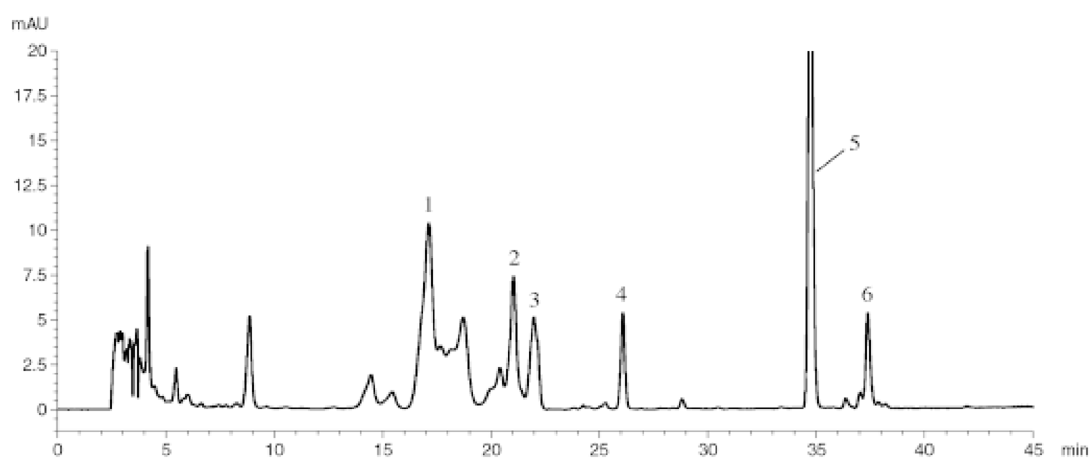


Figure 5 A reference fingerprint chromatogram of Radix Angelicae Pubescentis extract

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. 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 9.0%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 48.0%.
Ethanol-soluble extractives (cold extraction method): not less than 44.0%.
Ether-soluble extractives (carry out the method as indicated below):
Pulverize CMM sample, pass through a No. 4 sieve and mix well. Weigh accurately about 2.0 g of the powdered sample, previously dried over phosphorous pentoxide for 48 h, and transfer to a 100-mL conical flask. Add 70 mL of diethyl ether and several glass beads. Heat under reflux to slightly boil for 4 h, cool and filter. Wash the flask and residue with appropriate amount of ether. Combine the washings and filtrate, transfer to a 100-mL volumetric flask and make up to the mark with ether, mix well. Accurately transfer 50 mL of the solution to an evaporating dish, previously dried to constant weight. Expel the ether and dry it over phosphorous pentoxide for 24 h. Weigh immediately and accurately. The sample contains not less than 3.0% of ether-soluble extractives, calculated with reference to the dried substance.

7. ASSAY

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

Standard solution

Osthole standard stock solution, Std-Stock (44 mg/L)

Weigh accurately 2.2 mg of osthole CRS and dissolve in 50 mL of methanol.

Osthole standard solution for assay, Std-AS

Measure accurately the volume of the osthole Std-Stock, dilute with methanol to produce a series of solutions of 0.66, 4.40, 8.80, 13.20, 17.60 mg/L for osthole.

Test solution

Weigh accurately 0.1 g of the powdered sample and put into a 50-mL centrifugal tube, then add 25 mL of methanol. Sonicate (220 W) the mixture for 10 min. Centrifuge at about 3200 × *g* for 5 min. Filter and transfer the filtrate to a 100-mL volumetric flask. Repeat the extraction twice. Combine the extracts and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (320 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 water and acetonitrile (48:52, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections each with 20 µL of osthole Std-AS (4.40 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of osthole should not be more than 3.0%; the RSD of the retention time of osthole peak should not be more than 2.0%; the column efficiency determined from osthole peak should not be less than 10000 theoretical plates.

The *R* value between osthole 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 osthole Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of osthole against the corresponding concentrations of osthole Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify osthole peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of osthole Std-AS. The retention times of osthole 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

osthole in the test solution, and calculate the percentage content of osthole in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.50% of osthole ($C_{15}H_{16}O_3$), calculated with reference to the dried substance.