Rhapontici Radix

Figure 1 A photograph of Rhapontici Radix

A. Rhapontici Radix

B. Magnified image of transverse section of root

1. NAMES

Official Name: Rhapontici Radix

Chinese Name: 漏蘆

Chinese Phonetic Name: Loulu

2. SOURCE

Rhapontici Radix is the dried root of *Rhaponticum uniflorum* (L.) DC. (Asteraceae). The root is collected in spring and autumn, rootlets and soil removed, then dried under the sun to obtain Rhapontici Radix.

3. DESCRIPTION

Conical or flatted lumps, mostly twisted, varying in length, 10-25 mm in diameter. Externally greyish-brown to dark brown, rough, with longitudinal furrows and rhombic, netted clefts. Outer bark easily exfoliated. Root stock swollen, with the remains of stems and scaly leaf bases, top with greyish-white hairs. Light in weight, texture fragile, easily broken, fracture irregular, greyish-white to greyish-yellow, with greyish-black to brownish-black cleft in the centre. Odour characteristic; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis mostly fallen off. Metaderm consists of several to more than 20 layers of brown cell, cell walls slightly thickened, lignified and suberized. Secretory canals mainly scattered in parenchyma, varying in size, containing reddish-brown secretion, elliptical and larger secretory canals occasionally located near metaderm and arranged in irregularly interrupted ring. Phloem relatively broad, phloem rays wide. Cambium in a ring. Xylem vessels abundant, the large vessel groups often alternated with the small vessel groups. Xylem rays often scattered with radial clefts (Fig. 2).

Powder

Colour brown. Non-glandular hairs 10-31 μm in diameter, lignified. Metaderm cells subsquare or rectangular, walls slightly thickened, reddish-brown, lignified and suberized. Secretory canals 20-85 μm in diameter, containing reddish-brown secretions. Reticulate and bordered-pitted vessels abundant, 8-93 μm in diameter (Fig. 3).

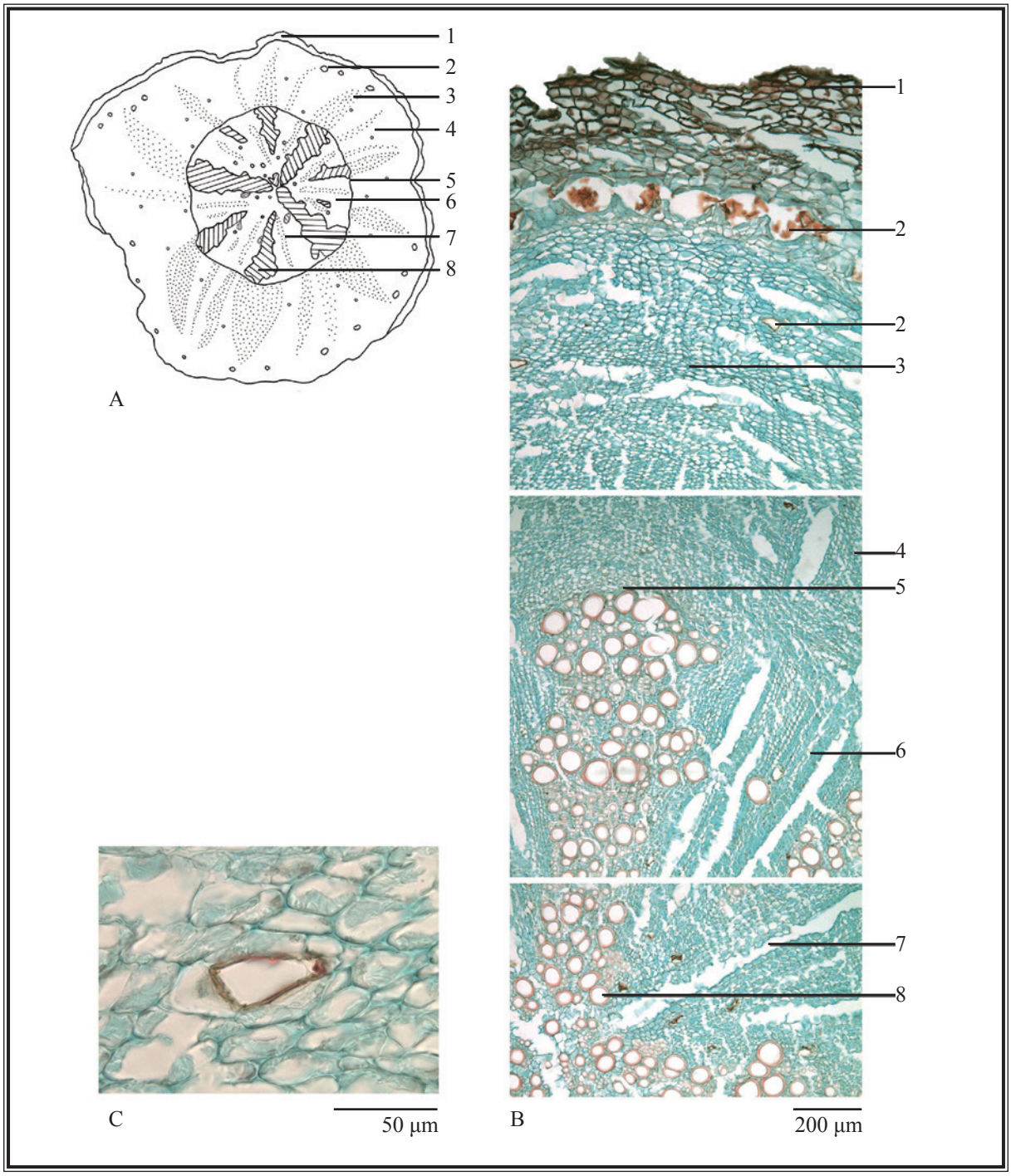

Figure 2 Microscopic features of transverse section of Rhapontici Radix

A. Sketch B. Section illustration C. Secretory canal

1. Metaderm 2. Secretory canal 3. Phloem 4. Phloem ray 5. Cambium
6. Xylem ray 7. Cleft 8. Xylem

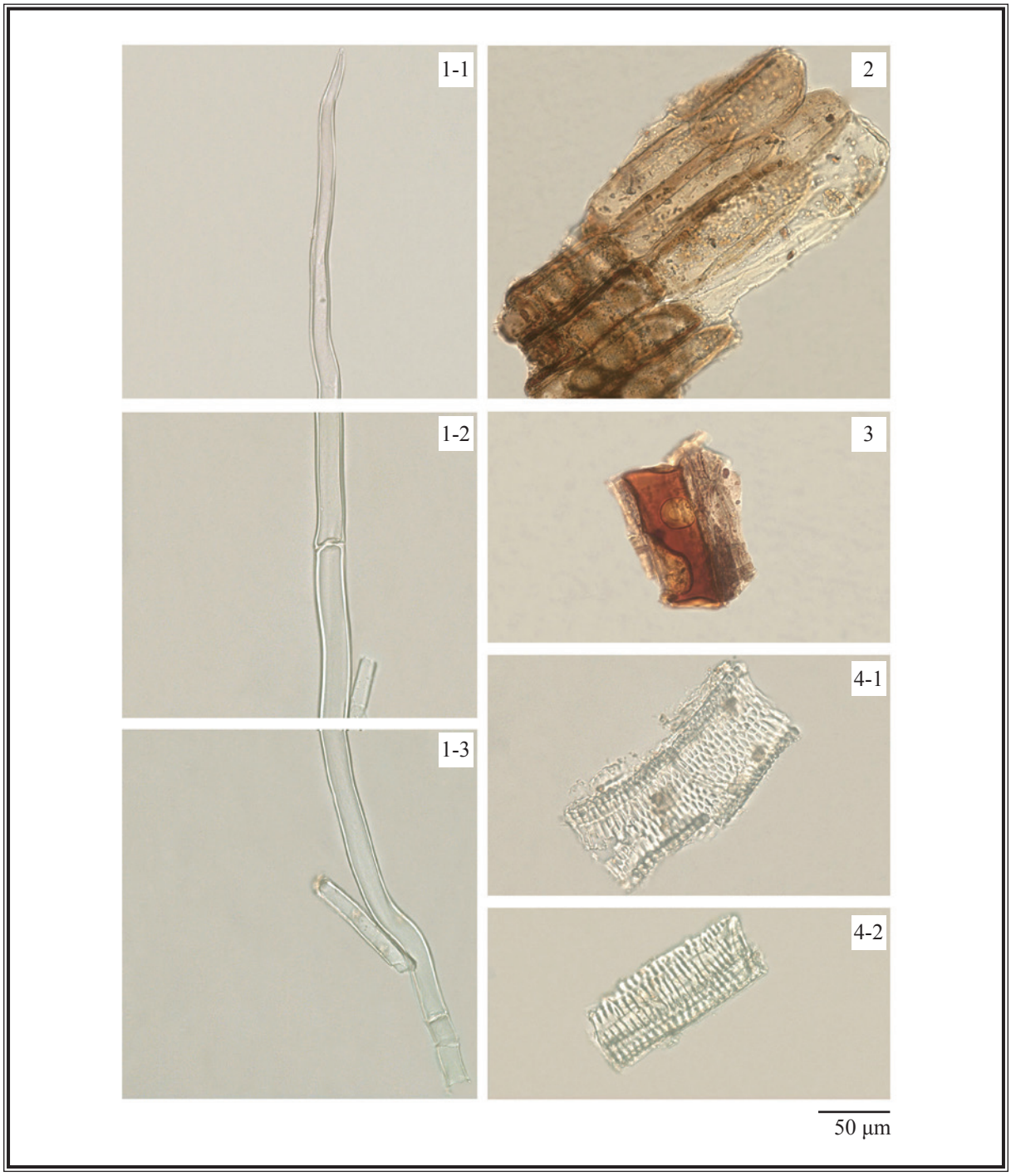

Figure 3 Microscopic features of powder of Rhapontici Radix (under the light microscope)

1. Non-glandular hair (1-1 upper part, 1-2 middle part, 1-3 lower part)
2. Metaderm cells 3. Secretory canal
4. Vessels (4-1 bordered-pitted vessel, 4-2 reticulate vessel)

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

Standard solution

β-Ecdysterone standard solution

Weigh 1.5 mg of β-ecdysterone CRS (Fig. 4) and dissolve in 5 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol (95%) and water (8:1:0.5, v/v).

Spray reagent

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (30%). Sonicate (270 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 (70%).

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 β-ecdysterone standard solution (1 μL) and the test solution (0.5 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. Spray the plate evenly with the spray reagent and heat at about 105ºC (about 10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

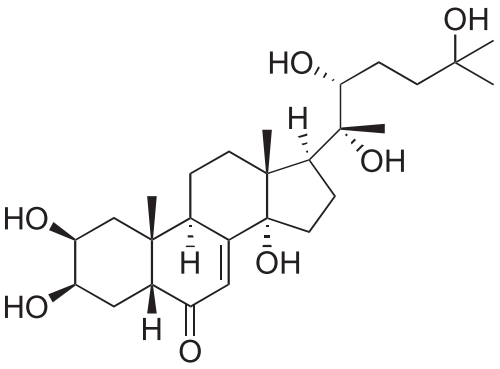

Figure 4 Chemical structure of β-ecdysterone

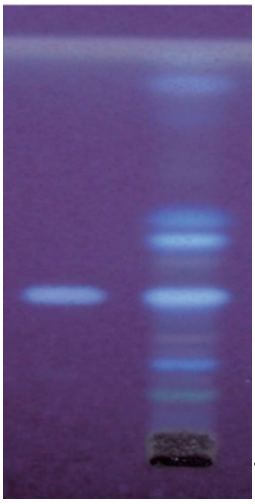

Figure 5 A reference HPTLC chromatogram of Rhapontici Radix extract observed under UV light (366 nm) after staining

1. β-Ecdysterone 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 β-ecdysterone (Fig. 5).

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

Standard solution

β-Ecdysterone standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 2.0 mg of β-ecdysterone CRS and dissolve in 10 mL of methanol (30%).

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (30%). 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 for one more time. Combine the filtrates and make up to the mark with methanol (30%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (247 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 – 30	90 → 80	10 → 20	linear gradient
30 – 60	80 → 60	20 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of β-ecdysterone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of β-ecdysterone should not be more than 5.0%; the RSD of the retention time of β-ecdysterone peak should not be more than 2.0%; the column efficiency determined from β-ecdysterone peak should not be less than 70000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject β-ecdysterone Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of β-ecdysterone peak in the chromatogram of β-ecdysterone Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify β-ecdysterone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of β-ecdysterone Std-FP. The retention times of β-ecdysterone 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 Rhapontici Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Rhapontici Radix extract

Peak No.	RRT	Acceptable Range
1	0.63	± 0.03
2	0.98	± 0.03
3 (marker, β-ecdysterone)	1.00	-
4	1.27	± 0.03
5	1.48	± 0.06

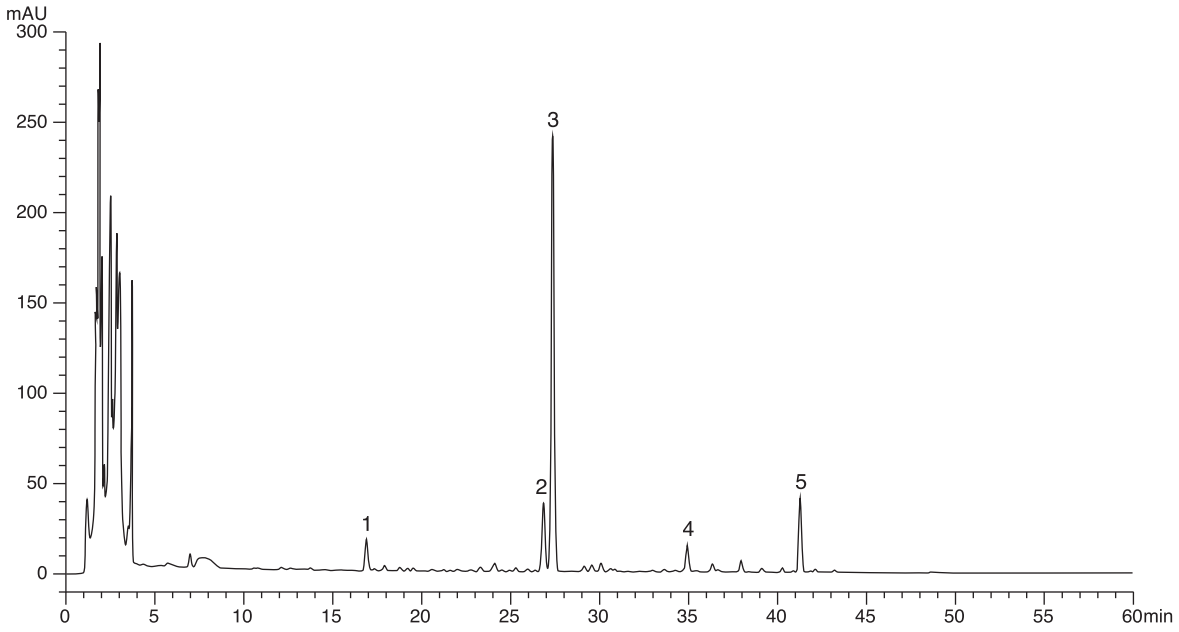

Figure 6 A reference fingerprint chromatogram of Rhapontici Radix extract

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 18.5%.

Acid-insoluble ash: not more than 4.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
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

β-Ecdysterone standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of β-ecdysterone CRS and dissolve in 5 mL of methanol (30%).

β-Ecdysterone standard solution for assay, Std-AS

Measure accurately the volume of the β-ecdysterone Std-Stock, dilute with methanol (30%) to produce a series of solutions of 10, 100, 200, 400, 600 mg/L for β-ecdysterone.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (30%). 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 for one more time. Combine the filtrates and make up to the mark with methanol (30%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (247 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 3) –

Table 3 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	90 → 80	10 → 20	linear gradient
30 – 60	80 → 60	20 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of β-ecdysterone Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of β-ecdysterone should not be more than 5.0%; the RSD of the retention time of β-ecdysterone peak should not be more than 2.0%; the column efficiency determined from β-ecdysterone peak should not be less than 70000 theoretical plates.

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

Limits

The sample contains not less than 0.18% of β-ecdysterone ($C_{27}H_{44}O_7$), calculated with reference to the dried substance**.**