Trichosanthis Pericarpium

Figure 1 A photograph of Trichosanthis Pericarpium

1. NAMES

Official Name: Trichosanthis Pericarpium

Chinese Name: 瓜蔞皮

Chinese Phonetic Name: Gualoupi

2. SOURCE

Trichosanthis Pericarpium is the dried ripe pericarp of *Trichosanthes kirilowii* Maxim. (Cucurbitaceae). The ripe fruit is collected in autumn, open the fruit, sarcocarp and seeds removed, then dried in a shaded area to obtain Trichosanthis Pericarpium.

3. DESCRIPTION

Usually cut into 2 to several pieces, edges involute, 6.9-14.3 cm long, relatively thick. Externally orange-red, orange-yellow or yellow, shrunken, sometimes with remnants of fruit stalk or stylopodium; inner surface yellowish-white. Texture relatively fragile, easily broken. Odour caramel-like; taste bland, slightly sour (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse Section

Exocarp consists of 1 layer of nearly squared cells, outer and lateral walls thickened. 12-15 layers of pigment cells beneath the exocarp, with thin wall. 2-5 layers of stone cells beneath the pigments cells layers. Parenchyma located in the inner side of stone cells, broad, scattered with numerous bicollateral vascular bundles. Xylem usually slightly bent outward, sometimes almost surrounding phloem on the outside (Fig. 2).

Powder

Colour pale yellowish-brown. Stone cells relatively numerous, scattered singly or several in groups, subsquare, rounded-polygonal, rectangular or oblong, 14-70 μm in diameter, up to 96 μm long, walls 4-17 μm thick, striations indistinct, pits small and dense, pit canals small and obvious. Epidermal cells of pericarp subsquare or subrectangular in section view, outer walls relatively thick, covered with cuticle, anticlinal walls inserted deep into epidermal cells as ridge-like; cells subsquare to subpolygonal in surface view, stomata anomocytic, subsidiary cells 5-7, occasionally found. Vessels mainly spiral, some with double-spiral or multi-spiral thickened, reticulate and bordered-pitted vessels also found. Lignified parenchymatous cells in pieces, some connected with stone cells, subpolygonal, subrounded or slightly irregular in shape, slightly lignified, pits relatively dense. Fibres scattered or in bundles, long stripe-shaped, pits oblique slit-shaped or cruciate-shaped, pit canals relatively obvious (Fig. 3).

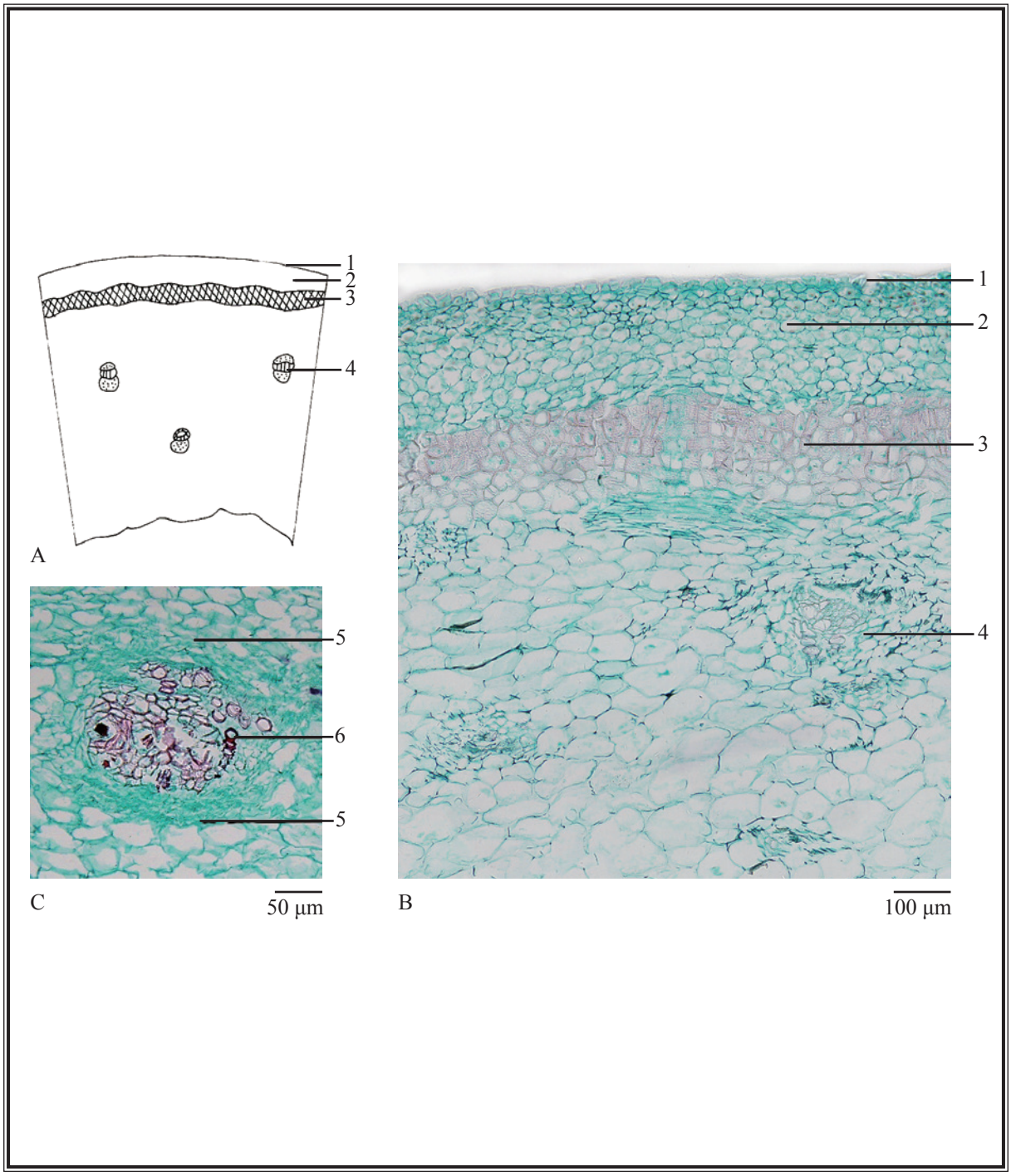

Figure 2 Microscopic features of transverse section of Trichosanthis Pericarpium

A. Sketch B. Section illustration C. Vascular bundle

1. Exocarp 2. Pigment cell layer 3. Stone cell layer 4. Vascular bundle 5. Phloem 6. Xylem

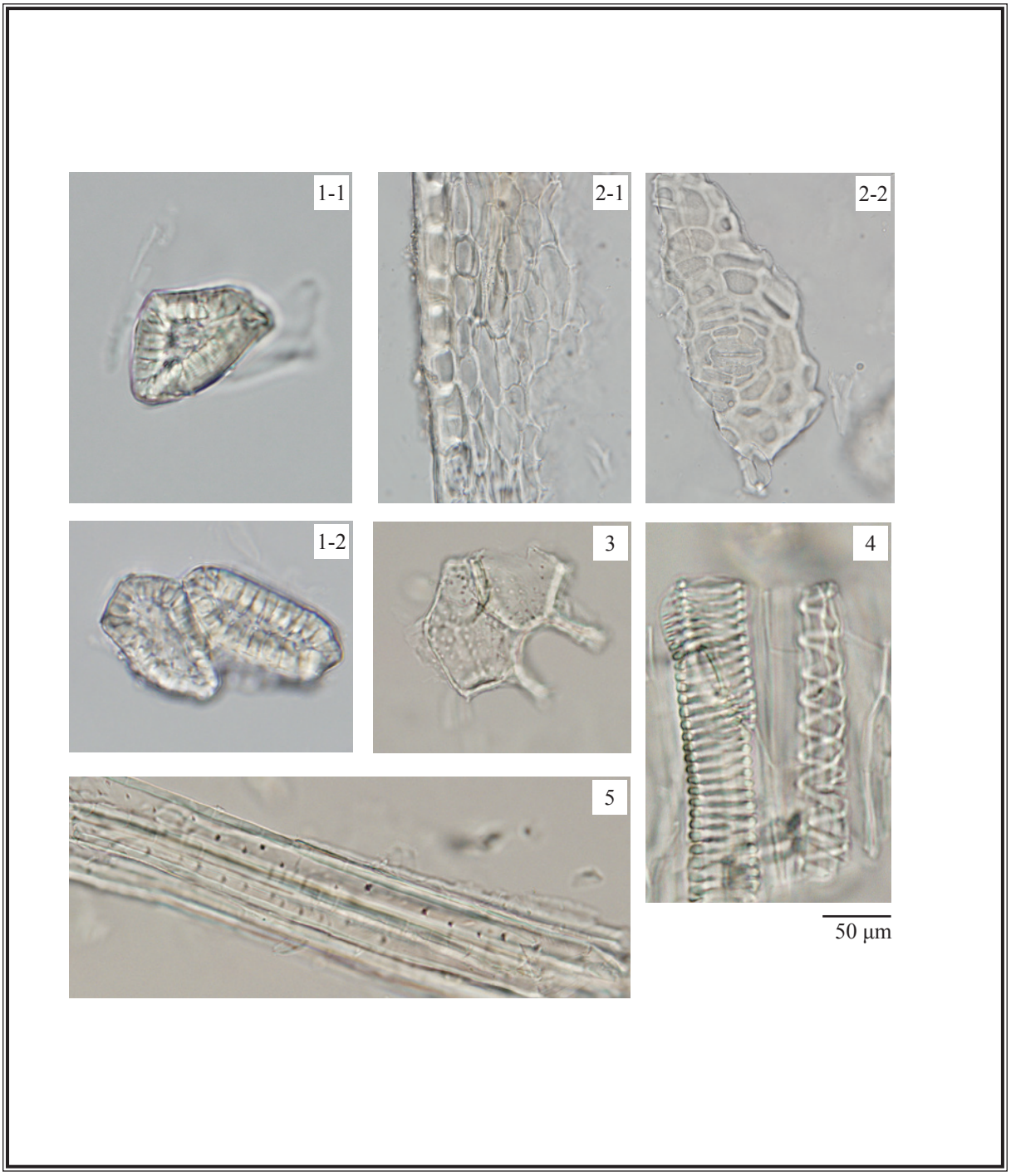

Figure 3 Microscopic features of powder of Trichosanthis Pericarpium (under the light microscope)

1. Stone cell (1-1 scattered singly, 1-2 several in groups)
2. Epidermal cells of pericarp (2-1 in section view, 2-2 in surface view)
3. Lignified parenchymatous cells 4. Vessels 5. Fibres

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

Standard solution

Spinasterol standard solution

Weigh 1.0 mg of spinasterol CRS (Fig. 4) and dissolve in 1 mL of ethyl acetate.

Developing solvent system

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

Spray reagent

Add slowly 5 mL of sulphuric acid to 95 mL of ethanol and dissolve 2.0 g of vanillin.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethyl acetate. 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 ethyl acetate. Filter through a 0.45-μm nylon filter.

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 spinasterol standard solution and the test solution (3 μL each) 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 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 until the spots or bands become visible (about 6 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

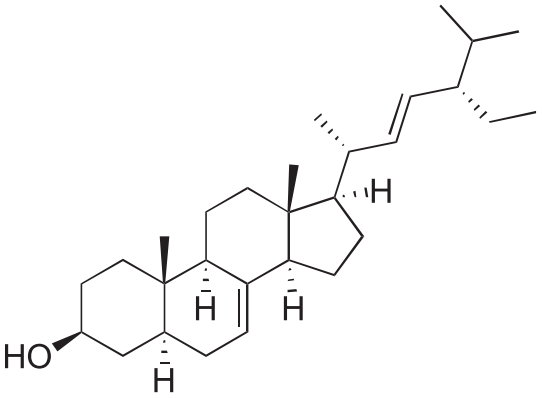

Figure 4 Chemical structure of spinasterol

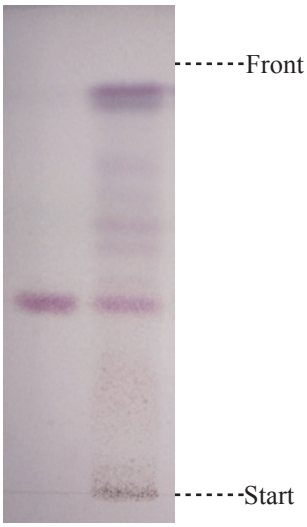

Figure 5 A reference HPTLC chromatogram of Trichosanthis Pericarpium extract observed under visible light after staining

1. Spinasterol 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 spinasterol (Fig. 5).

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

Standard solution

Spinasterol standard solution for fingerprinting, Std-FP (140 mg/L)

Weigh 1.4 mg of spinasterol CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of cyclohexane. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (211 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.6 mL/min. The mobile phase is a mixture of methanol and water (99:1, v/v). The elution time is about 50 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of spinasterol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of spinasterol should not be more than 5.0%; the RSD of the retention time of spinasterol peak should not be more than 2.0%; the column efficiency determined from spinasterol peak should not be less than 8000 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 spinasterol Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of spinasterol peak in the chromatogram of spinasterol Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify spinasterol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of spinasterol Std-FP. The retention times of spinasterol 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 three characteristic peaks of Trichosanthis Pericarpium extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Trichosanthis Pericarpium extract

Peak No.	RRT	Acceptable Range
1	0.67	± 0.03
2	0.78	± 0.03
3 (marker, spinasterol)	1.00	-

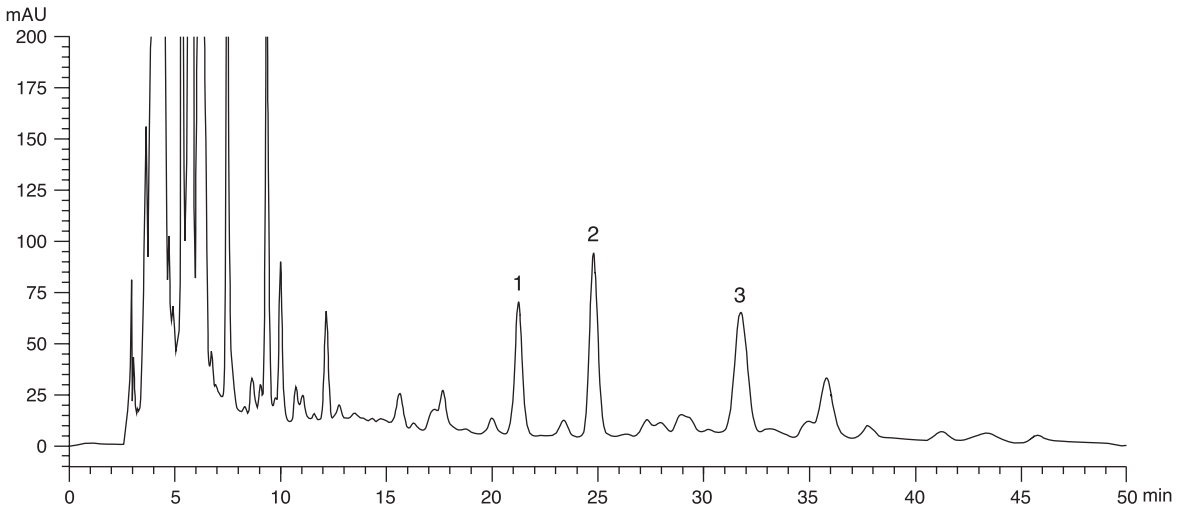

Figure 6 A reference fingerprint chromatogram of Trichosanthis Pericarpium extract

For positive identification, the sample must give the above three 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 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 39.0%.
Ethanol-soluble extractives (hot extraction method): not less than 31.0%.

7. ASSAY

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

Standard solution
Spinasterol standard stock solution, Std-Stock (900 mg/L)
Weigh accurately 9.0 mg of spinasterol CRS and dissolve in 10 mL of methanol.
Spinasterol standard solution for assay, Std-AS
Measure accurately the volume of the spinasterol Std-Stock, dilute with methanol to produce a series of solutions of 45, 63, 90, 135, 270 mg/L for spinasterol.

Test solution
Weigh accurately 2.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 50 mL of cyclohexane. Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm RC filter.

Chromatographic system
The liquid chromatograph is equipped with a DAD (211 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.6 mL/min. The mobile phase is a mixture of methanol and water (99:1, v/v). The elution time is about 50 min.

System suitability requirements

Perform at least five replicate injections, each using 20 µL of spinasterol Std-AS (90 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of spinasterol should not be more than 5.0%; the RSD of the retention time of spinasterol peak should not be more than 2.0%; the column efficiency determined from spinasterol peak should not be less than 8000 theoretical plates.

The *R* value between spinasterol 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 spinasterol Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of spinasterol against the corresponding concentrations of spinasterol 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 spinasterol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of spinasterol Std-AS. The retention times of spinasterol 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 spinasterol in the test solution, and calculate the percentage content of spinasterol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.024% of spinasterol ($C_{29}H_{48}O$), calculated with reference to the dried substance.