Tinosporae Radix

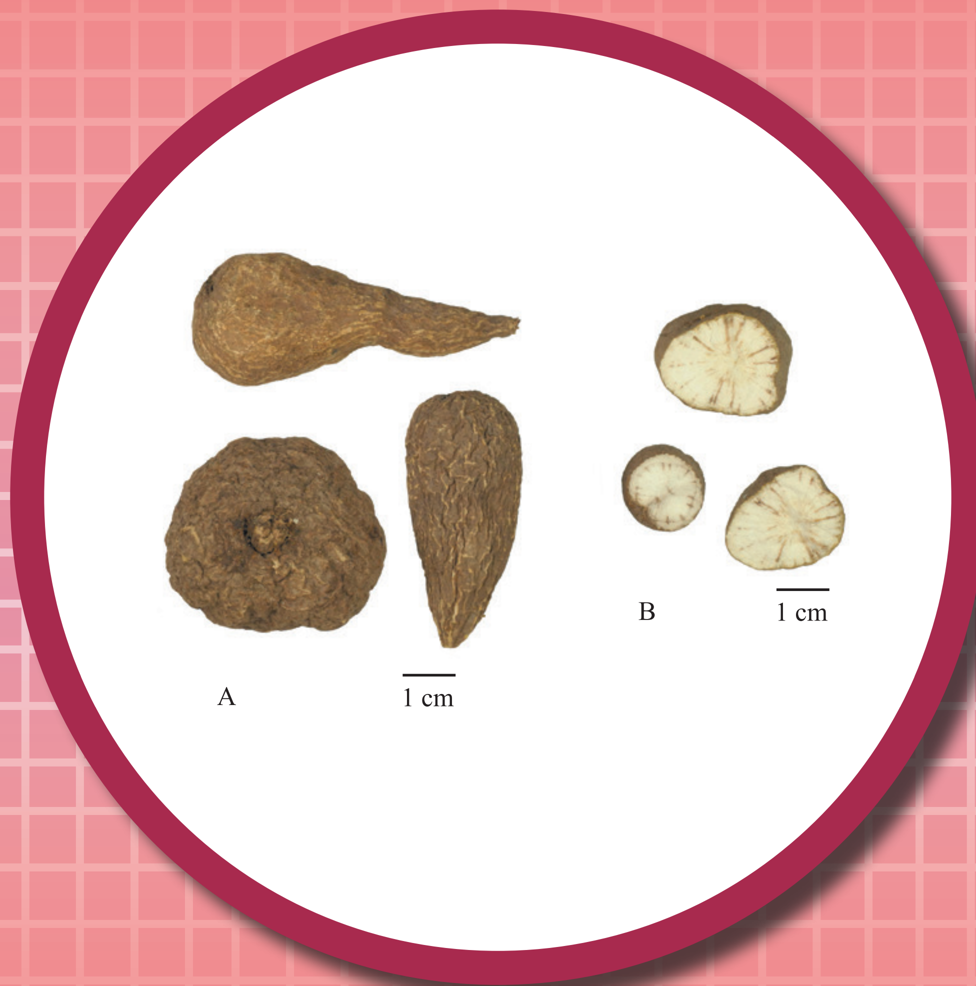

Figure 1 A photograph of Tinosporae Radix

A. Tinosporae Radix B. Transverse section of root tubers

1. NAMES

Official Name: Tinosporae Radix

Chinese Name: 金果欖

Chinese Phonetic Name: Jinguolan

2. SOURCE

Tinosporae Radix is the dried root tuber of *Tinospora sagittata* (Oliv.) Gagnep. (Menispermaceae). The root tuber is collected in autumn and winter, rootlets removed, washed clean, then dried under the sun or baked at 45-50ºC to obtain Tinosporae Radix.

3. DESCRIPTION

Irregular tuber, 2.5-8.5 cm long, 13-45 mm in diameter. Externally yellowish-brown, brown to greyish-brown, rugged, usually with shallow or deep wrinkles. Texture hard, uneasily broken. Fracture yellowish-white, showing radial striations deeper in colour. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of 3-14 layers of cells. Cortex narrow. Pericycle consists of 2-4 layers of stone cells, arranged in a ring, lumen contains prisms of calcium oxalate. Phloem narrow. Cambium in a ring. Xylem narrow and long, mostly occupying 5/6 portion of the diameter of the root tuber; xylem vessels surrounded by fibres, arranged radially emanating from the centre of the tuber. Parenchyma filled with starch granules (Fig. 2).

Powder

Colour yellowish-white to yellowish-brown. Stone cells scattered singly or 2-8 in groups, subrounded or elliptic, 21-57 µm in diameter; lumens contain 1-8 prism(s) of calcium oxalate, 5-22 µm in diameter; stone cells bright white to yellow, prisms of calcium oxalate polychromatic under the polarized microscope. Cork cells in pieces, pale yellowish-brown to yellowish-brown, polygonal or subsquare. Starch granules abundant, mainly simple starch granules, subrounded, semicircular, helmet-shaped or irregularly rounded, 8-31 µm in diameter, striations mostly indistinct; black and cruciate-shaped under the polarized microscope. Vessels mostly bordered-pitted, 15-47 µm in diameter. Fibres with thickened walls, pit apertures distinct; bright white under the polarized microscope (Fig. 3).

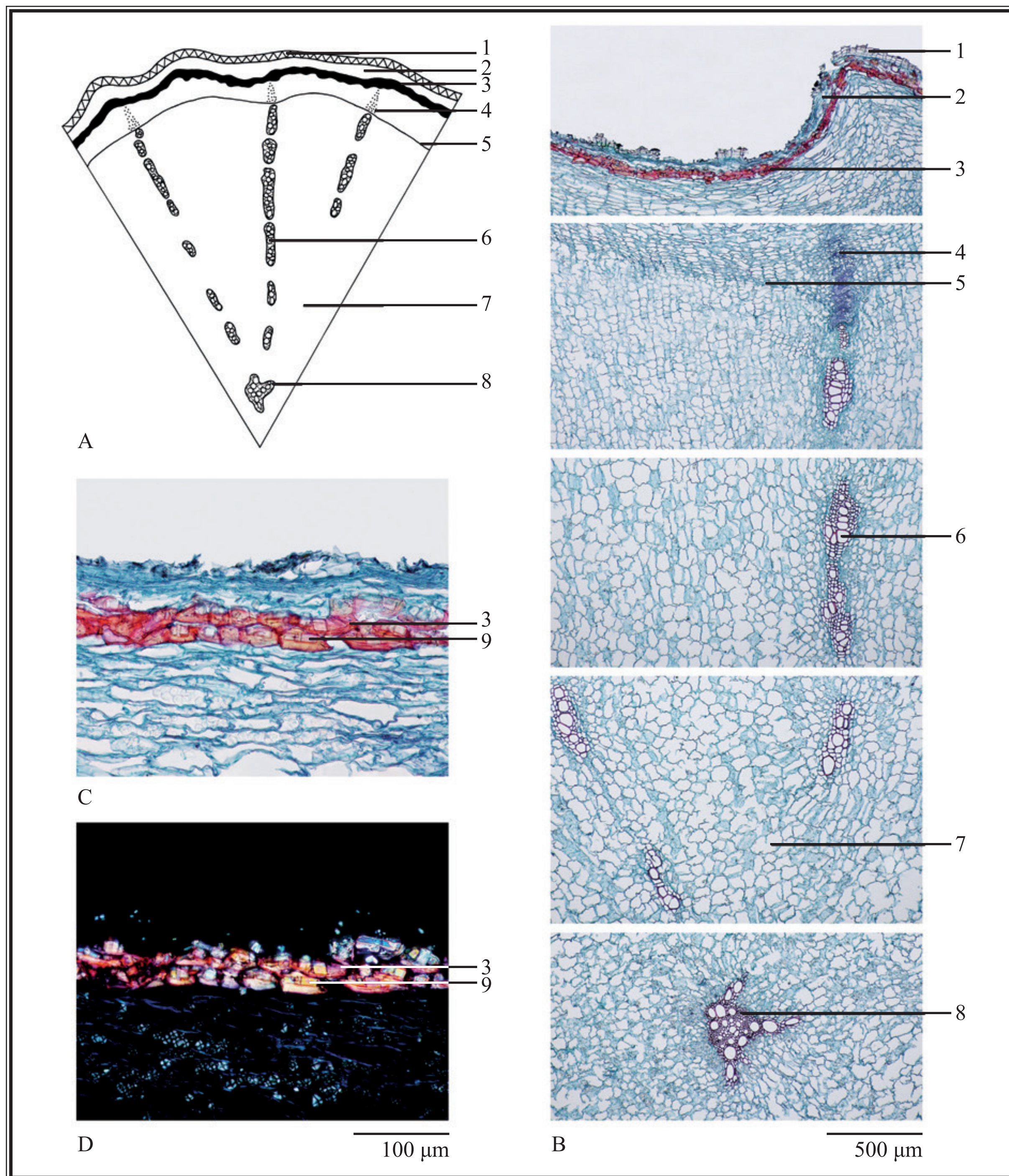

Figure 2 Microscopic features of transverse section of Tinosporae Radix

A. Sketch B. Section illustration C. Pericycle (under the light microscope)
D. Pericycle (under the polarized microscope)

1. Cork 2. Cortex 3. Stone cell 4. Phloem 5. Cambium 6. Xylem 7. Parenchyma 8. Fibre
9. Prism of calcium oxalate

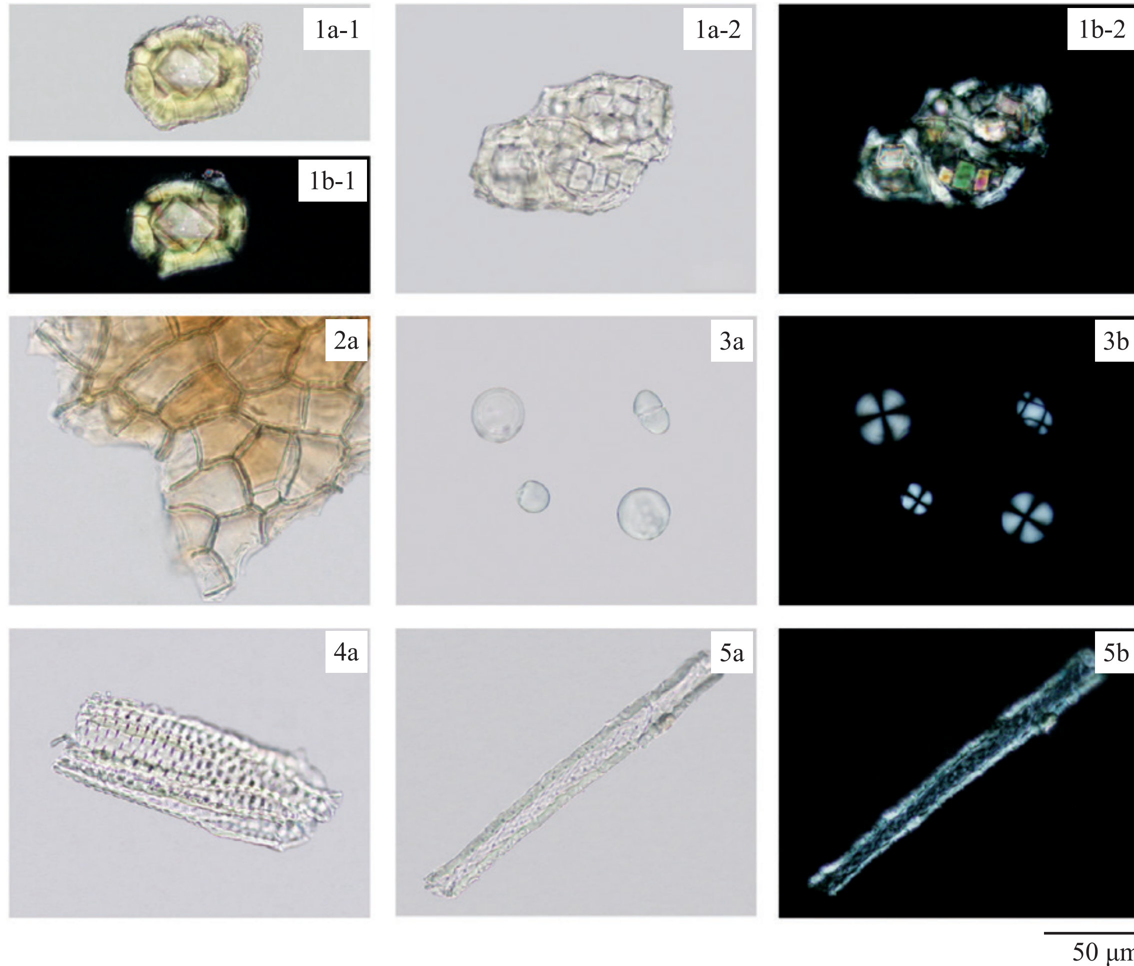

Figure 3 Microscopic features of powder of Tinosporae Radix

1. Stone cells contain prism(s) of calcium oxalate
(1-1 contain a prism of calcium oxalate, 1-2 contain several prisms of calcium oxalate)
2. Cork cells 3. Starch granules 4. Vessels 5. Fibre

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

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

Standard solution

Columbin standard solution

Weigh 1.0 mg of columbin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (28%, v/v), methanol, ethyl acetate and *n*-hexane (1:6:9:10, 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 10 mL of methanol. Sonicate (400 W) the mixture for 30 min. 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 columbin standard solution and the test solution (2 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 (about 2 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

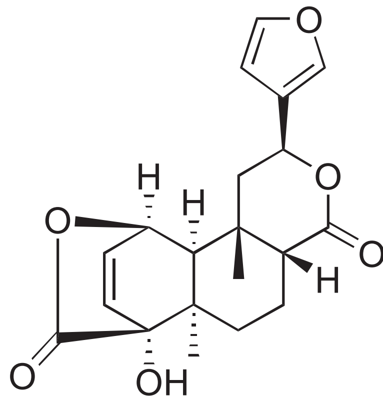

Figure 4 Chemical structure of columbin

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

1. Columbin 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 columbin (Fig. 5).

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

Standard solution

Columbin standard solution for fingerprinting, Std-FP (32 mg/L)

Weigh 0.32 mg of columbin CRS and dissolve in 10 mL of methanol (75%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (75%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (3.5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	85 → 57	15 → 43	linear gradient
35 – 40	57 → 44	43 → 56	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of columbin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of columbin should not be more than 5.0%; the RSD of the retention time of columbin peak should not be more than 2.0%; the column efficiency determined from columbin peak should not be less than 100000 theoretical plates.

The *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

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

Peak No.	RRT	Acceptable Range
1	0.30	± 0.03
2	0.43	± 0.03
3	0.77	± 0.03
4	0.98	± 0.03
5 (marker, columbin)	1.00	-

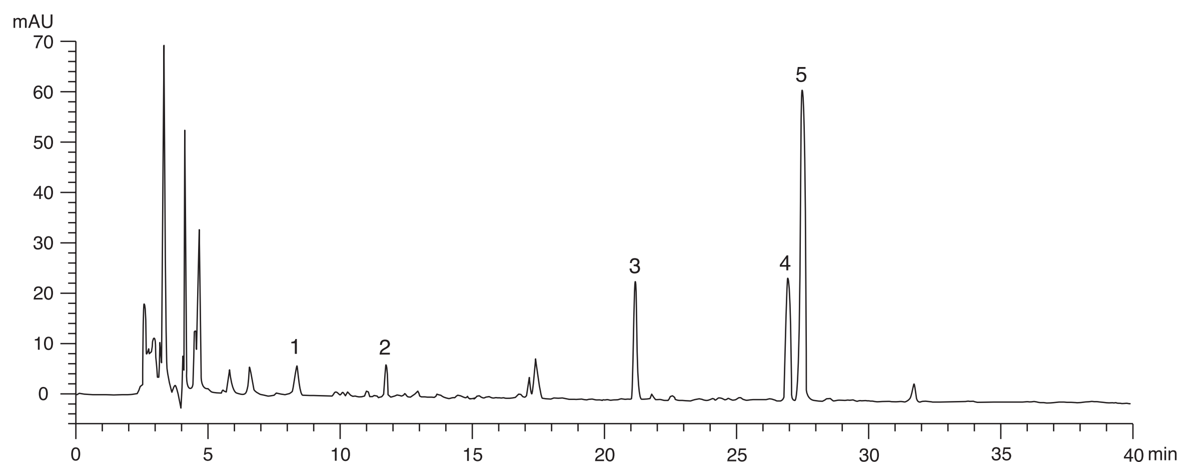

Figure 6 A reference fingerprint chromatogram of Tinosporae Radix extract

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Columbin standard stock solution, Std-Stock (250 mg/L)

Weigh accurately 2.5 mg of columbin CRS and dissolve in 10 mL of methanol (75%).

Columbin standard solution for assay, Std-AS

Measure accurately the volume of the columbin Std-Stock, dilute with methanol (75%) to produce a series of solutions of 8, 16, 32, 64, 128 mg/L for columbin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (75%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (3.5 µm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.05% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 35	85 → 57	15 → 43	linear gradient
35 – 40	57 → 44	43 → 56	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of columbin Std-AS (32 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of columbin should not be more than 5.0%; the RSD of the retention time of columbin peak should not be more than 2.0%; the column efficiency determined from columbin peak should not be less than 100000 theoretical plates.

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

Limits

The sample contains not less than 1.0% of columbin ($C_{20}H_{22}O_6$), calculated with reference to the dried substance.