Dioscoreae Bulbiferae Rhizoma

Figure 1 A photograph of Dioscoreae Bulbiferae Rhizoma

A. Dioscoreae Bulbiferae Rhizoma
B. Magnified image of cut surface (showing granular fracture)
C. Magnified image of cut surface (showing orange-yellow spots)

1. NAMES

Official name: Dioscoreae Bulbiferae Rhizoma

Chinese name: 黃藥子

Chinese phonetic name: Huangyaozi

2. SOURCE

Dioscoreae Bulbiferae Rhizoma is the dried tuber of *Dioscorea bulbifera* L. (Dioscoreaceae). The tuber is collected in autumn, rootlets, residual stems and leaves removed, washed clean, cut into slices when fresh, then dried under the sun to obtain Dioscoreae Bulbiferae Rhizoma.

3. DESCRIPTION

Mostly transversely cut thick slices, rounded or subrounded, 14-143 mm in diameter, 0.1-1.9 cm in thickness. Cork easily exfoliated, externally brownish-black, shrunken, white punctiform protuberances scars of rootlets numerous, occasionally with remains of curved rootlets. Transversely cut surface yellowish-white to yellowish-brown, flat or bumpy. Texture hard and fragile. Fracture granular, scattered with orange-yellow spots. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of cells, arranged densely, with relatively thick wall, slightly lignified. Stone cells arranged in an interrupted ring. Secretory canal located at the outer part of the basal tissue. Parenchymatous cells in basal tissue loosely arranged, containing abundant starch granules. Mucilage cells numerous, containing raphides of calcium oxalate. Vascular bundles few, collateral and scattered (Fig. 2).

Powder

Colour brownish-yellow or greyish-yellow. Starch granules numerous, singly scattered or in group, mainly simple starch granules, oblong, ovoid or irregular-triangular, 6-82 μm in diameter, hilum pointed or stellate, located at the smaller end, usually not obvious; black and cruciated-shaped under the polarized microscope. Mucilage cells contain raphides of calcium oxalate, 14-122 μm long; polychromatic under the polarized microscope. Stone cells pale yellow to brownish-yellow, mostly singly scattered or 2-5 in groups, long elliptical, subrounded, fusiform or irregular in shape, with striations, distinct pits and pit canals; yellowish-white under the polarized microscope. Vessels mainly reticulate and spiral, 8-40 μm in diameter. Cork cells subpolygonal or subrectangular, walls slightly thick, occasionally found. Secretory canals mostly broken, containing reddish-brown secretions (Fig. 3).

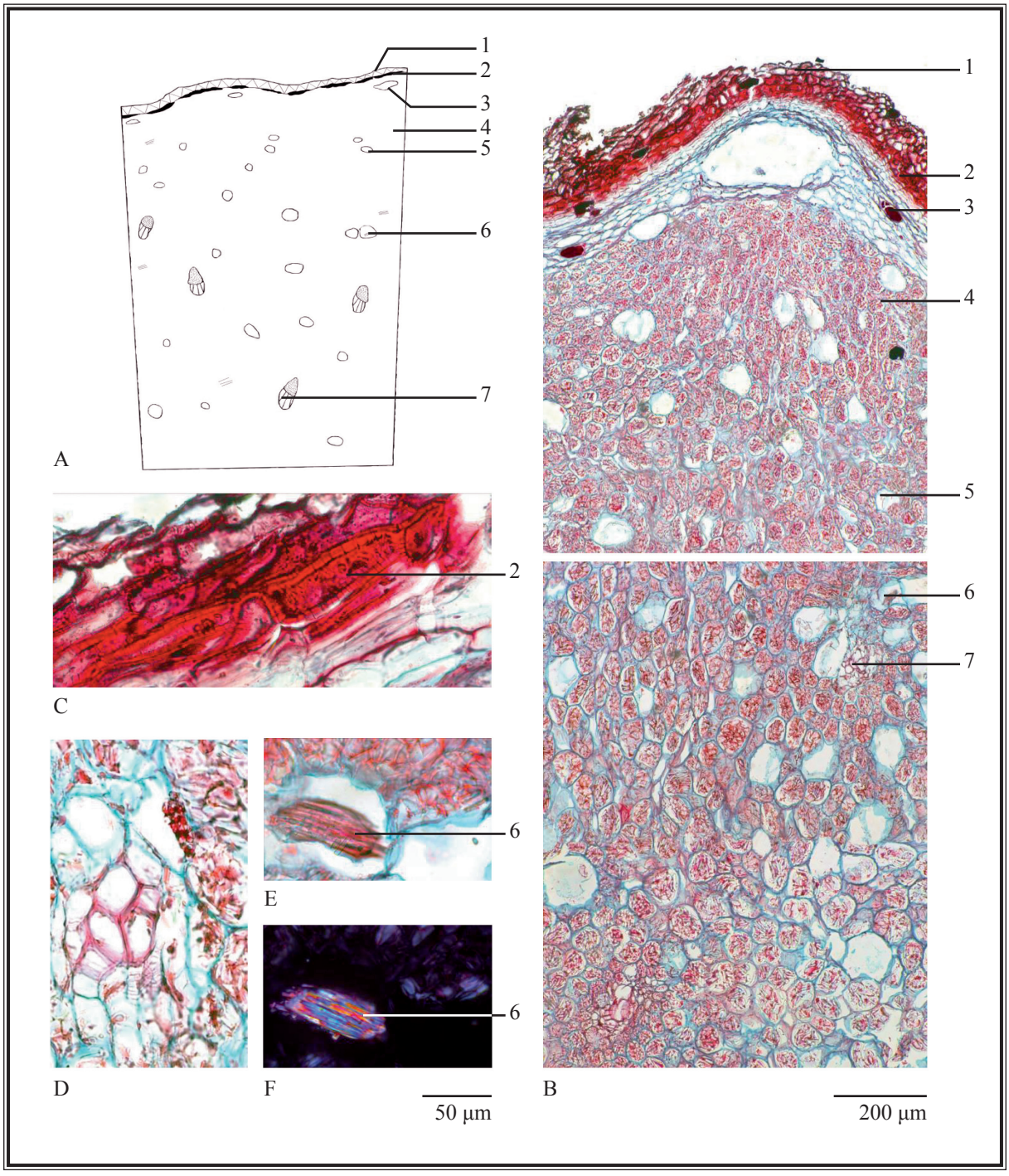

Figure 2 Microscopic features of transverse section of Dioscoreae Bulbiferae Rhizoma

A. Sketch B. Section illustration C. Stone cells
D. Vascular bundle
E. Raphides of calcium oxalate (under the light microscope)
F. Raphides of calcium oxalate (under the polarized microscope)

1. Cork 2. Stone cell 3. Secretory canal 4. Basal tissue 5. Mucilage cells
6. Raphides of calcium oxalate 7. Vascular bundle

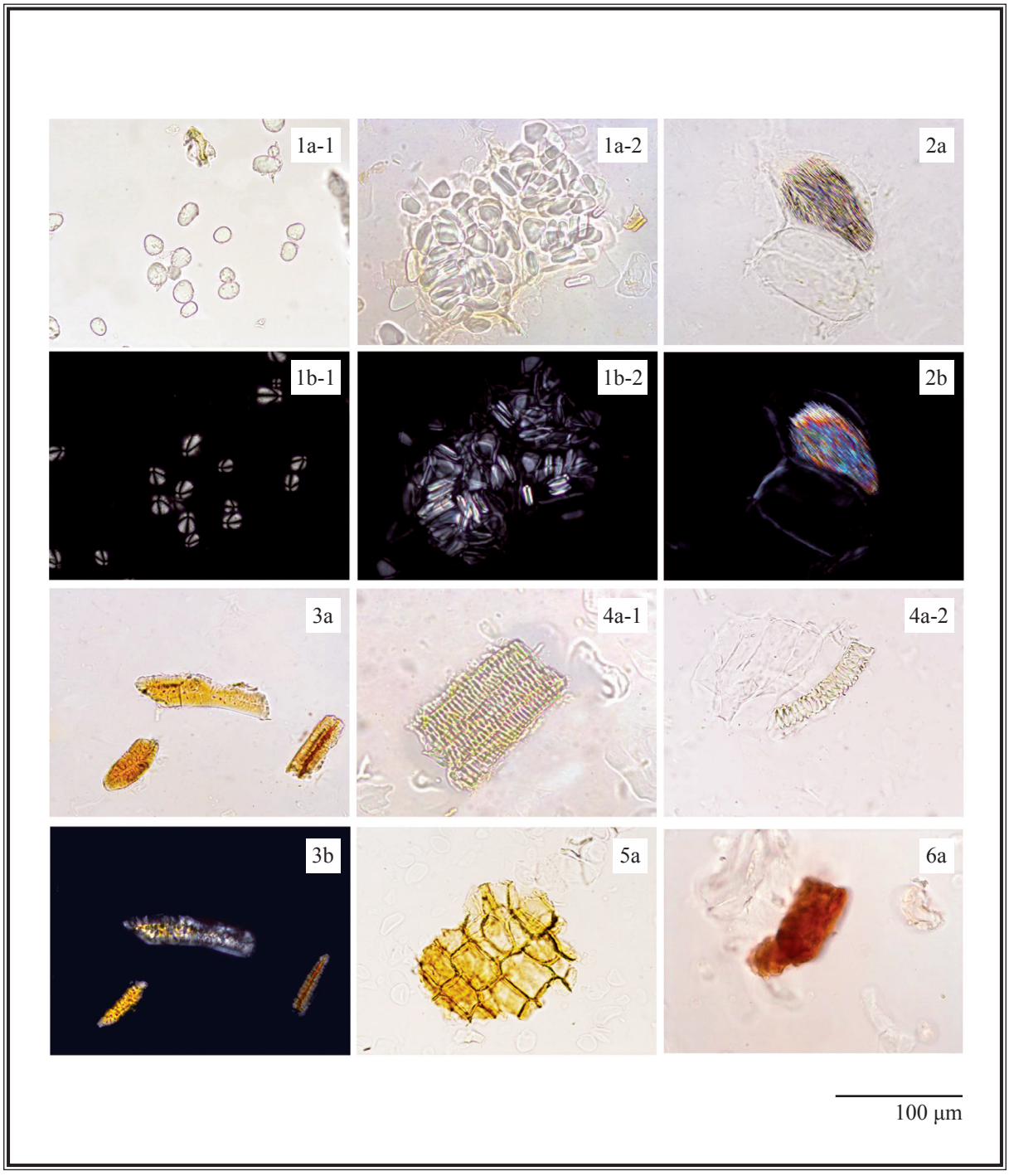

Figure 3 Microscopic features of powder of Dioscoreae Bulbiferae Rhizoma

1. Starch granules (1-1 singly scattered, 1-2 in group)
2. Mucilage cell with raphides of calcium oxalate 3. Stone cells
4. Vessels (4-1 reticulate vessels, 4-2 spiral vessel) 5. Cork cells 6. Secretory canal

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

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

Standard solution

Diosbulbin B standard solution

Weigh 0.5 mg of diosbulbin B CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate and methanol (10:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol and dissolve 10 g of vanillin.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately diosbulbin B standard solution (3 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 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 3 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

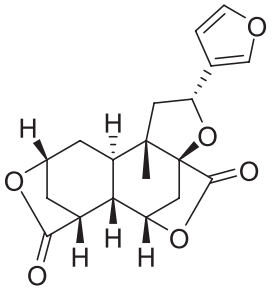

Figure 4 Chemical structure of diosbulbin B

Figure 5 A reference HPTLC chromatogram of Dioscoreae Bulbiferae Rhizoma extract observed under visible light after staining

1. Diosbulbin B 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 diosbulbin B (Fig. 5).

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

Standard solution

Diosbulbin B standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of diosbulbin B CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (300 W) the mixture for 1 h. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction one more time. Combine the filtrates. Wash the residue with methanol. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the extract to a 20-mL volumetric flask 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 DAD (210 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 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 – 25	95 → 80	5 → 20	linear gradient
25 – 40	80 → 50	20 → 50	linear gradient
40 – 50	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of diosbulbin B Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of diosbulbin B should not be more than 5.0%; the RSD of the retention time of diosbulbin B peak should not be more than 2.0%; the column efficiency determined from diosbulbin B peak should not be less than 380000 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 diosbulbin B Std-FP and the test solution (5 µL each) into the HPLC system and record the chromatograms. Measure the retention time of diosbulbin B peak in the chromatogram of diosbulbin B Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify diosbulbin B peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of diosbulbin B Std-FP. The retention times of diosbulbin B 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 Dioscoreae Bulbiferae Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Dioscoreae Bulbiferae Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.50	± 0.03
2	0.57	± 0.03
3 (marker, diosbulbin B)	1.00	-

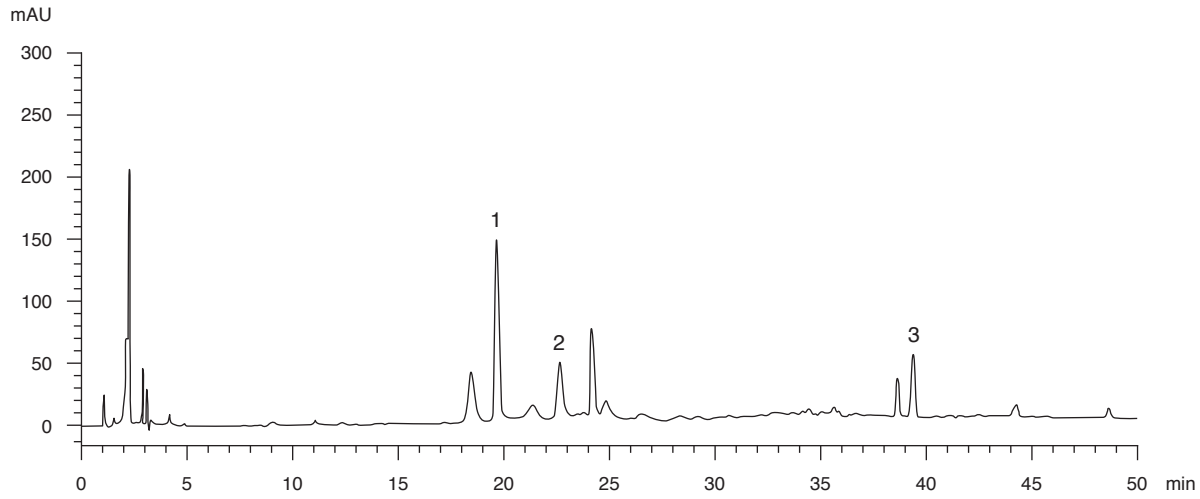

Figure 6 A reference fingerprint chromatogram of Dioscoreae Bulbiferae Rhizoma extract

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 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 0.5%.

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 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 5.0%.

7. ASSAY

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

Standard solution

Diosbulbin B standard stock solution, Std-Stock (400 mg/L)

Weigh accurately 4.0 mg of diosbulbin B CRS and dissolve in 10 mL of methanol.

Diosbulbin B standard solution for assay, Std-AS

Measure accurately the volume of the diosbulbin B Std-Stock, dilute with methanol to produce a series of solutions of 3, 25, 100, 200, 300 mg/L for diosbulbin B.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of methanol. Sonicate (300 W) the mixture for 1 h. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction one more time. Combine the filtrates. Wash the residue with methanol. Combine the extracts. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the extract to a 20-mL volumetric flask 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 DAD (210 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 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 – 25	95 → 80	5 → 20	linear gradient
25 – 40	80 → 50	20 → 50	linear gradient
40 – 50	50	50	isocratic

System suitability requirements

Perform at least five replicate injections, each using 5 μL of diosbulbin B Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of diosbulbin B should not be more than 5.0%; the RSD of the retention time of diosbulbin B peak should not be more than 2.0%; the column efficiency determined from diosbulbin B peak should not be less than 380000 theoretical plates.

The *R* value between diosbulbin B peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of diosbulbin B Std-AS (5 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of diosbulbin B against the corresponding concentrations of diosbulbin B 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 diosbulbin B peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of diosbulbin B Std-AS. The retention times of diosbulbin B 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 diosbulbin B in the test solution, and calculate the percentage content of diosbulbin B in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.082% of diosbulbin B ($C_{19}H_{20}O_6$), calculated with reference to the dried substance.

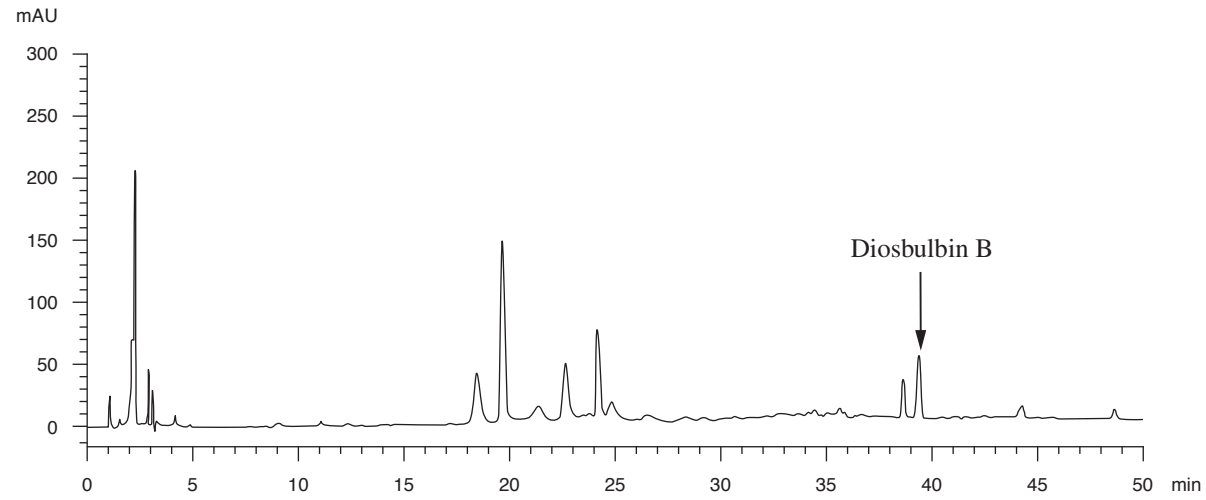

Figure 7 A reference assay chromatogram of Dioscoreae Bulbiferae Rhizoma extract