Fritillariae Hupehensis Bulbus

Figure 1 A photograph of Fritillariae Hupehensis Bulbus

1. NAMES

Official Name: Fritillariae Hupehensis Bulbus

Chinese Name: 湖北貝母

Chinese Phonetic Name: Hubeibeimu

2. SOURCE

Fritillariae Hupehensis Bulbus is the dried bulb of *Fritillaria hupehensis* Hsiao et K. C. Hsia (Liliaceae). The bulb is collected in early summer when the plant withers. After removed of soil, the bulb is soaked in saturated calcium hydroxide (0.15%) or washed clean, then dried under the sun to obtain Fritillariae Hupehensis Bulbus.

3. DESCRIPTION

Oblate, 8-37 mm in diameter. The outer surface off-white to pale brown, the outer scale leaves 2, fleshy, slightly reniform in shape, or varying considerably in size; the large scale closely embracing the small one, and enclosing 2-6 small scales and the remains of dried shrunken stem. The inner surface white to pale yellow. Base depressed, with remains of epidermis and a few fibrous roots. The single scale leaf reniform-shoe-shaped, 0.7-3.5 cm high, 4-18 mm in diameter. Texture fragile, easily broken, fracture whitish, starchy. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer epidermis of scale leaves consists of 3-5 layers of cells, inner epidermis consists of 2-4 layers of cells, covered with cuticle. Parenchymatous cells filled with starch granules. Crystals of calcium oxalate occasionally visible in epidermal cells. Vessels small, scattered in parenchyma (Fig. 2).

Powder

Colour off-white to pale brown. Starch granules fairly abundant, broadly ovoid, long ellipsoid or subspheroid, 11-70 μm in diameter, hilum pointed, V-shaped or slit-shaped, striations distinct;

compound granules rare, composed of 2-3 units; black and cruciate in shape under the polarized microscope. Crystals of calcium oxalate subsquare, rhombic, granular or clustered, 20-117 μm in diameter; polychromatic under the polarized microscope. Spiral vessels, 6-32 μm in diameter. Epidermal cells square or polygonal, anticlinal walls irregularly beaded, 25-75 μm in diameter; stoma occasionally visible, oblate, with 4-5 subsidiary cells (Fig. 3).

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

Standard solution

Hupehenine standard solution

Weigh 1.0 mg of hupehenine CRS (Fig. 4) and dissolve in 4 mL of ethanol.

Developing solvent system

Prepare a mixture of dichloromethane, acetone and diethylamine (8:2:0.5, v/v).

Spray reagent

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

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 2 mL of ammonium hydroxide solution (25%, v/v) and 10 mL of ethanol. Sonicate (90 W) the mixture for 15 min. Filter the mixture.

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 hupehenine standard solution (2 μL) and the test solution (10 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 8.5 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 105°C until the spots or bands become visible (about 3 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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 hupehenine.

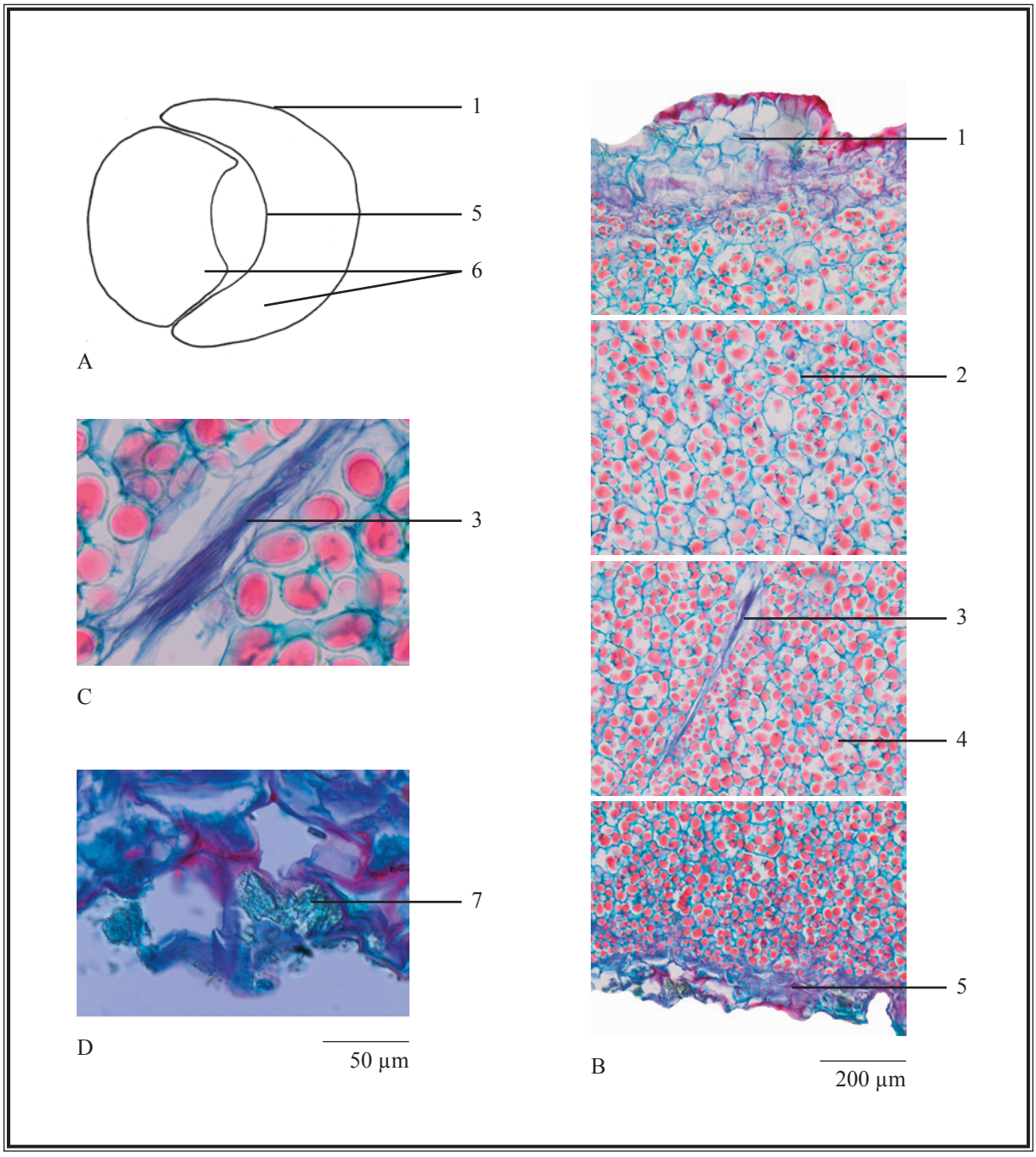


Figure 2 Microscopic features of transverse section of Fritillariae Hupehensis Bulbus

A. Sketch B. Section illustration of scale leaf C. Vessels D. Crystals of calcium oxalate

1. Outer epidermis 2. Parenchyma 3. Vessels 4. Starch granules
5. Inner epidermis 6. Scale leaf 7. Crystals of calcium oxalate

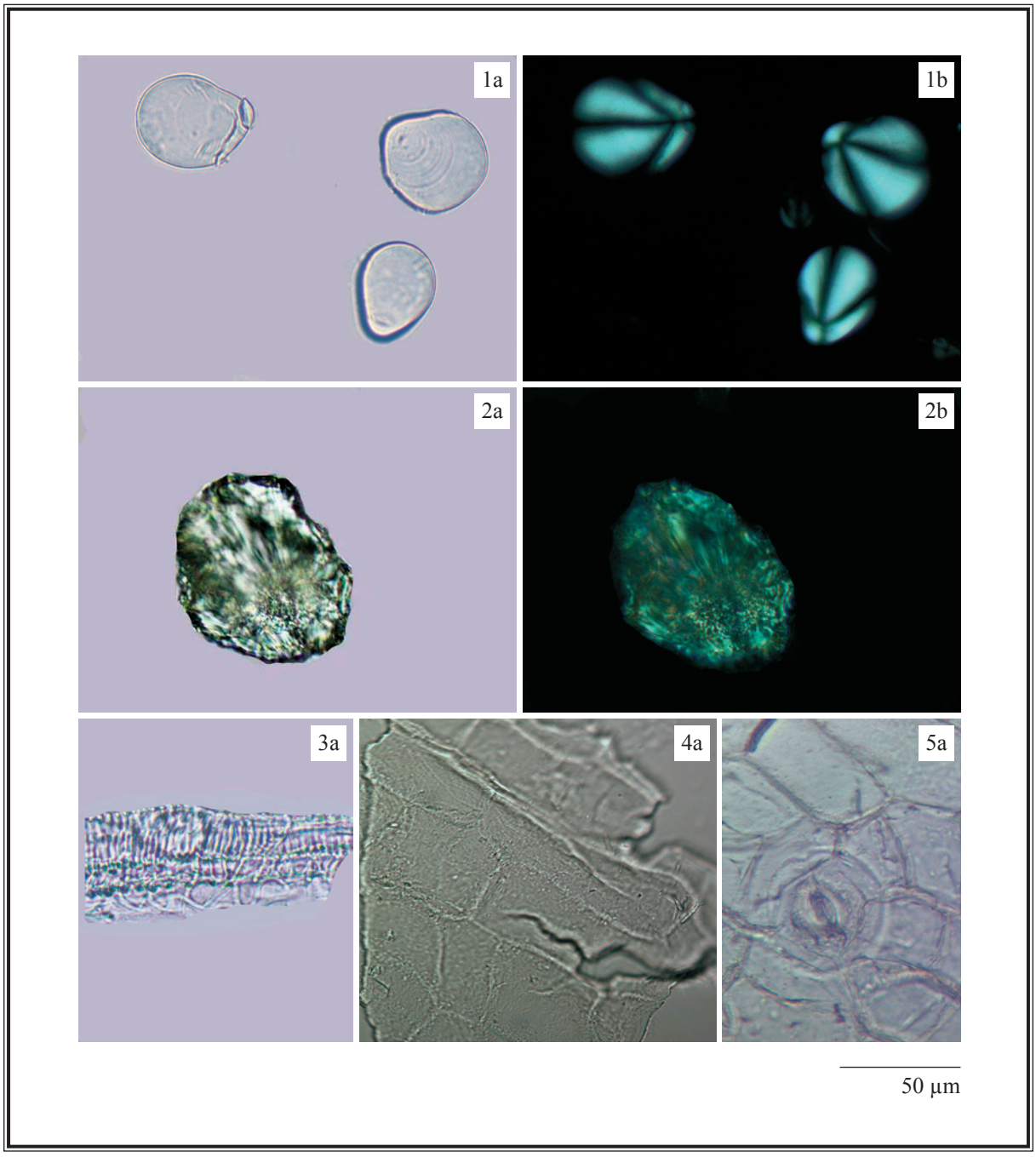
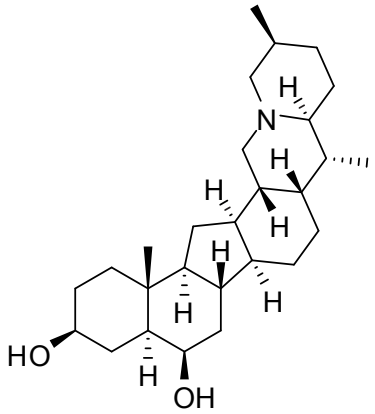


Figure 3 Microscopic features of powder of Fritillariae Hupehensis Bulbus

1. Starch granules 2. Crystal of calcium oxalate 3. Vessels 4. Epidermal cells 5. Stoma

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

(i)



(ii)

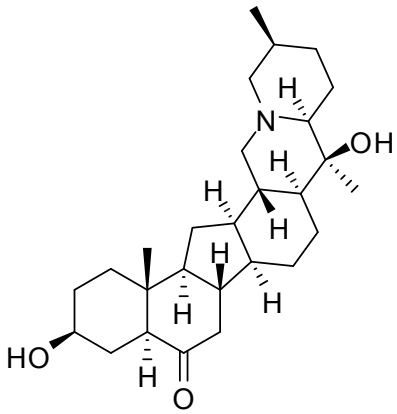


Figure 4 Chemical structures of (i) hupehenine and (ii) peiminine

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

Standard solution

Peiminine standard solution for fingerprinting, Std-FP (480 mg/L)

Weigh 0.96 mg of peiminine CRS (Fig. 4) and dissolve in 2 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 1 h. Add 20 mL of a mixture of dichloromethane and acetone (1:2, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Filter and 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 nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 88°C; nebulizer gas (N_2) flow: 0.8 L/min] 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)	0.02% Triethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	40 → 5	60 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of peiminine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of peiminine should not be more than 5.0%; the RSD of the retention time of peiminine peak should not be more than 2.0%; the column efficiency determined from peiminine peak should not be less than 10000 theoretical plates.

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

Procedure

Separately inject peiminine Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of peiminine peak in the chromatogram of peiminine Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify peiminine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of peiminine Std-FP. The retention times of peiminine 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 four characteristic peaks of Fritillariae Hupehensis Bulbus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Fritillariae Hupehensis Bulbus extract

Peak No.	RRT	Acceptable Range
1	0.81	± 0.03
2 (marker, peiminine)	1.00	-
3	1.21	± 0.03
4 (hupehenine)	1.56	± 0.04

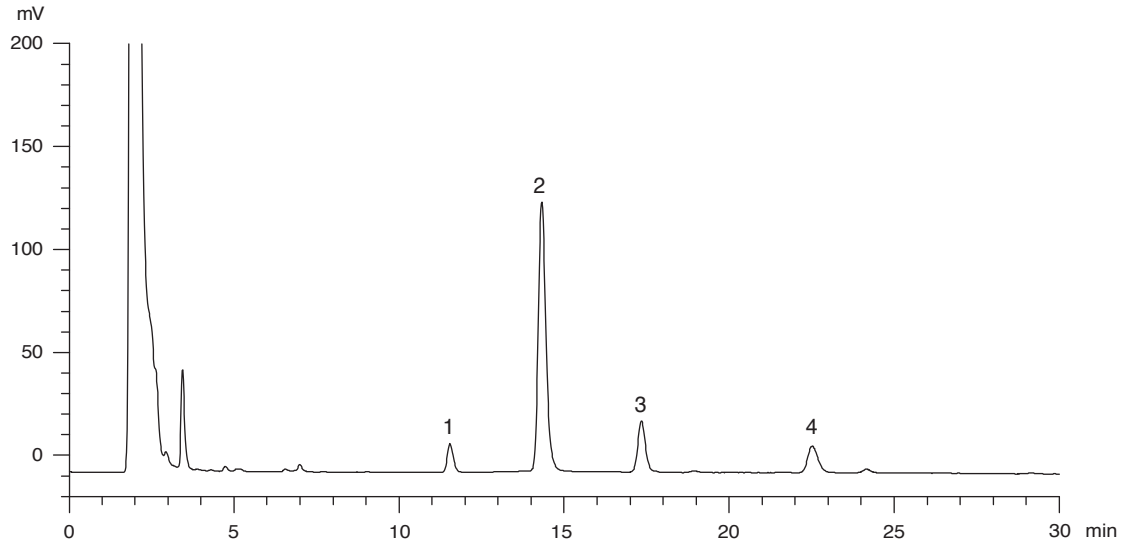


Figure 5 A reference fingerprint chromatogram of Fritillariae Hupehensis Bulbus extract

For positive identification, the sample must give the above four 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 XVIII)*: 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 10.0%.
Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Peiminine standard stock solution, Std-Stock (2400 mg/L)

Weigh accurately 4.8 mg of peiminine CRS and dissolve in 2 mL of methanol.

Peiminine standard solution for assay, Std-AS

Measure accurately the volume of the peiminine Std-Stock, dilute with methanol to produce a series of solutions of 120, 240, 360, 600, 900 mg/L for peiminine.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 1 h. Add 20 mL of a mixture of dichloromethane and acetone (1:2, v/v). Reflux the mixture for 30 min. Cool down to room temperature. Filter and 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 nylon filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 88 °C; nebulizer gas (N_2) flow: 0.8 L/min] 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)	0.02% Triethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	40 → 5	60 → 95	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of peiminine Std-AS (360 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of peiminine should not be more than 5.0%; the RSD of the retention time of peiminine peak should not be more than 2.0%; the column efficiency determined from peiminine peak should not be less than 10000 theoretical plates.

The *R* value between peiminine 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 peiminine Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of peiminine against the natural logarithm of the corresponding concentrations of peiminine 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 peiminine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of peiminine Std-AS. The retention times of peiminine peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of peiminine in the test solution by using the following equation –

$$\text{Concentration of peiminine in the test solution} = e^{[\text{Ln (A)}-I]/m}$$

Where A = the peak area of peiminine in the test solution,

I = the y-intercept of the 5-point calibration curve of peiminine,

m = the slope of the 5-point calibration curve of peiminine.

Calculate the percentage content of peiminine in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.16% of peiminine ($C_{27}H_{43}NO_3$), calculated with reference to the dried substance.