Bulbus Fritillariae Thunbergii

Figure 1 A photograph of Bulbus Fritillariae Thunbergii
1. Dabei 2. Zhubei

1. NAMES

Official Name: Bulbus Fritillariae Thunbergii

Chinese Name: 浙貝母

Chinese Phonetic Name: Zhebeimu

2. SOURCE

Bulbus Fritillariae Thunbergii is the dried bulb of *Fritillaria thunbergii* Miq. (Liliaceae). The bulb is collected in early summer when the plant withers, washed clean, sorted according to size, the central bud is removed from the larger bulbs (commonly known as "Dabei"), but not from the smaller ones (commonly known as "Zhubei"), and the outer scale leaf removed by crushing. Afterwards, the crushed bulb mixed with calcinated shells powder (to absorb the juice that dashes out), and then dried to obtain Bulbus Fritillariae Thunbergii.

3. DESCRIPTION

Dabei: Bulb enclosed by an outer single scale leaf, slightly crescent-shaped, 1.1-2.1 cm high, 10-45 mm in diameter. The outer surface whitish to pale yellow, the inner surface white or pale brown, covered with white powder. Texture hard and fragile, easily broken; fracture white to yellowish-white, highly starchy. Odour slight; taste slightly bitter (Fig. 1).

Zhubei: Whole bulb oblate, 0.9-2.1 cm high, 11-35 mm in diameter. Externally whitish, the 2 outer scale leaves plump and fleshy, slightly reniform in shape, held to each other, enclosing 2-3 small scale leaves and the remains of the dried shrunken stem (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermis of scale leaves consists of 3-7 rows of cells, lower epidermis of 2-4 rows of cells, their outer wall thickened with cuticle. Occasional crystals of calcium oxalate are visible in the epidermal cells. Vessels small, scattered in the parenchyma tissue. Parenchyma cells replete with starch granules (Fig. 2).

Powder

Colour greyish-white. Starch granules numerous. Simple granules ovoid to broadly ovoid or ellipsoid, 5-52 μm in diameter; hilum pointed, cleft-like or V-shaped in the narrowed end, striations visible. Compound granules rare. Crystals of calcium oxalate rare, minute, mostly granular, some have fusiform, square or thin bacilliform shape. Vessels mostly spiral, 3-16 μm in diameter. Epidermal cells subpolygonal or rectangular, anticlinal wall slightly crooked and beaded-thickened; stomata less visible, subrounded or depressed-rounded, 50-58 μm in diameter, with 4-5 subsidiary cells (Fig. 3).

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

Standard solutions

Peimine standard solution

Weigh 2.0 mg of peimine CRS (Fig. 4) and dissolve in 2 mL of ethyl acetate.

Peiminine standard solution

Weigh 2.0 mg of peiminine CRS (Fig. 4) and dissolve in 2 mL of ethyl acetate.

Developing solvent system

Prepare a mixture of ammonium hydroxide (25%, v/v), petroleum ether (60-80°C) and acetone (0.2:10:10, v/v).

Spray reagents

Solution A

Weigh 0.85 g of bismuth subnitrate and dissolve in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

Solution B

Weigh 4 g of potassium iodide and dissolve in 10 mL of water.

Spray reagent 1

Transfer 5 mL of Solution A, 5 mL of Solution B and 20 mL of glacial acetic acid to a 100-mL volumetric flask and make up to the mark with water. Freshly prepare the reagent.

Spray reagent 2

Weigh 5 g of sodium nitrite and dissolve in 100 mL of water.

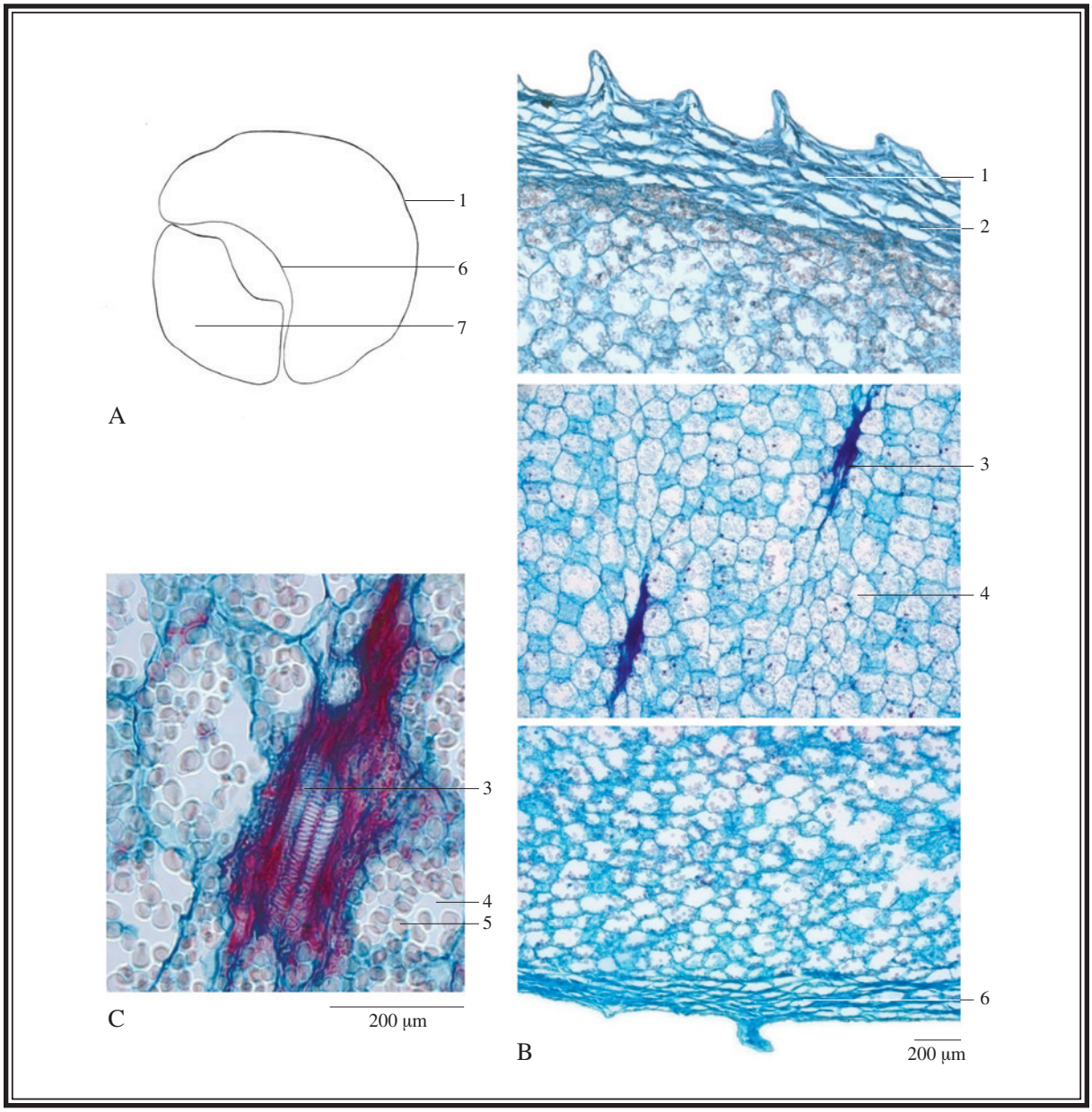


Figure 2 Microscopic features of transverse section of Bulbus Fritillariae Thunbergii

A. Sketch B. Section illustration C. Vessels and parenchyma cells

1. Upper epidermis 2. Crystals of calcium oxalate 3. Vessels
4. Parenchyma cell 5. Starch granule 6. Lower epidermis 7. Scale leaf

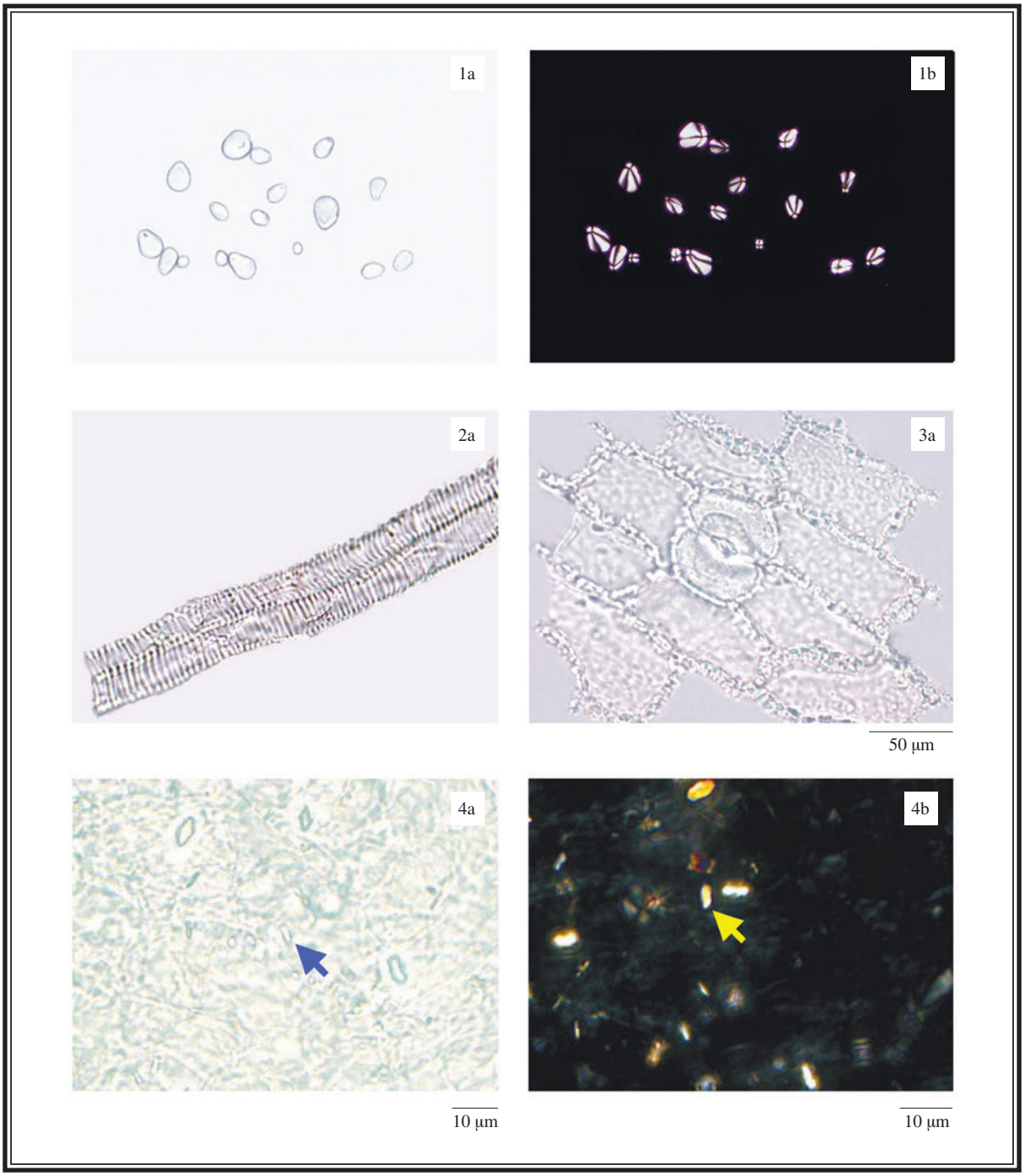


Figure 3 Microscopic features of powder of Bulbus Fritillariae Thunbergii

1. Starch granules 2. Vessels 3. Epidermal cells and a stoma
4. Crystals of calcium oxalate

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

Test solution

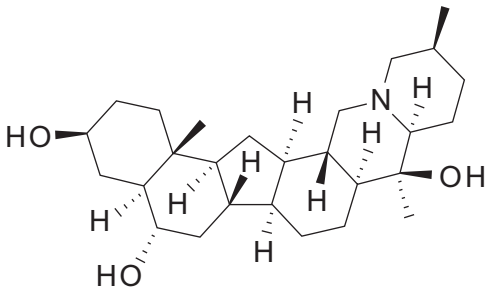
Weigh 5.0 g of the powdered sample and place it in a 50-mL conical flask, then add 2 mL of ammonium hydroxide (25%, v/v) and 20 mL of ethyl acetate. Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethyl acetate.

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 peimine standard solution, peiminine standard solution (2 μL each) and the test solution (4 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 1 and the spray reagent 2. Dry the plate in air until the spots or bands become visible (about 1 - 3 min). Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of peimine and peiminine.

(i)



(ii)

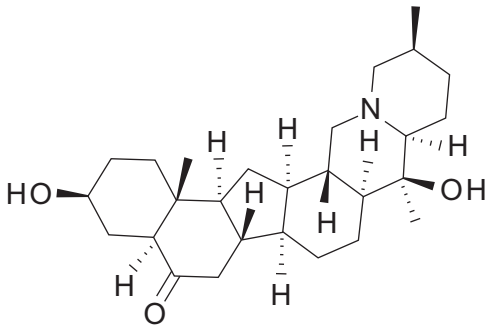


Figure 4 Chemical structures of (i) peimine and (ii) peiminine

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

Standard solution

Peimine standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 1.0 mg of peimine CRS and dissolve in 20 mL of ethanol (50%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL test tube, then add 10 mL of ethanol (50%). Macerate the mixture for 2 h. Sonicate (560 W) the mixture for 1 h. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 116°C; nebulizer gas (N_2) flow: 3.2 L/min] and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	100→90	0→10	linear gradient
20–60	90→20	10→80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of peimine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of peimine should not be more than 5.0%; the RSD of the retention time of peimine peak should not be more than 2.0%; the column efficiency determined from peimine peak should not be less than 400000 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 peimine Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of peimine peak in the chromatogram of peimine Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify peimine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of peimine Std-FP. The retention times of peimine

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 Bulbus Fritillariae Thunbergii extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.70	±0.03
2 (marker, peimine)	1.00	-
3	1.03	±0.03
4	1.07	±0.03

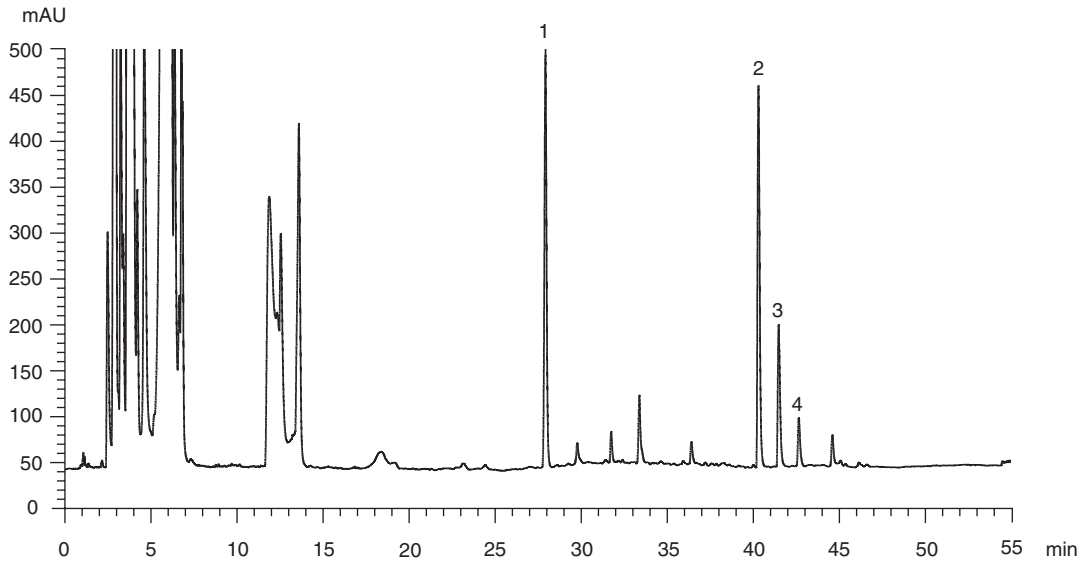


Figure 5 A reference fingerprint chromatogram of Bulbus Fritillariae Thunbergii extract

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 XV)*: 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)*: not more than 18.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (hot extraction method): not less than 9.0%.

7. ASSAY

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

Standard solution

Mixed peimine and peiminine standard stock solution, Std-Stock (250 mg/L each)

Weigh accurately 2.5 mg of peimine CRS and 2.5 mg of peiminine CRS, and dissolve in 10 mL of ethanol (50%).

Mixed peimine and peiminine standard solution for assay, Std-AS

Measure accurately the volume of the mixed peimine and peiminine Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 5, 10, 20, 50, 150 mg/L for both peimine and peiminine.

Test solution

Weigh accurately 2.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Macerate the mixture for 1 h. Sonicate (560 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter. Transfer the solution to a 50-mL volumetric flask. Repeat the extraction for two more times each with 10 mL of ethanol (50%). Filter the supernatant with the same RC filter. Transfer the solution to the volumetric flask and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 85°C; nebulizer gas (N_2) flow: 2.2 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. The mobile phase is a mixture of triethylamine (1%, v/v) and acetonitrile (30:70, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed peimine and peiminine Std-AS (50 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of peimine and peiminine should not be more than 5.0%; the RSD of the retention times of peimine peak and peiminine peak should not be more than 2.0%; the column efficiencies determined from peimine peak and peiminine peak should not be less than 10000 theoretical plates.

The *R* value between peimine peak and peiminine peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of the mixed peimine and peiminine Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of peimine and peiminine against the natural logarithm of the corresponding concentrations of the mixed peimine and peiminine Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify peimine peak and peiminine peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed peimine and peiminine Std-AS. The retention times of peimine peaks and peiminine peaks in both chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of peimine and peiminine in the test solution by using the following equation –

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

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

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

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

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

Limits

The sample contains not less than 0.079% of the total content of peimine ($C_{27}H_{45}NO_3$) and peiminine ($C_{27}H_{43}NO_3$), calculated with reference to the dried substance.