Bulbus Fritillariae Ussuriensis

Figure 1　A photograph of Bulbus Fritillariae Ussuriensis

1. NAMES

Official Name: Bulbus Fritillariae Ussuriensis

Chinese Name: 平貝母

Chinese Phonetic Name: Pingbeimu

2. SOURCE

Bulbus Fritillariae Ussuriensis is the dried bulb of *Fritillaria ussuriensis* Maxim. (Liliaceae). The bulb is collected in the spring, fibrous roots and soil removed, washed clean, then dried under the sun to obtain Bulbus Fritillariae Ussuriensis.

3. DESCRIPTION

Oblate in shape, 0.5-1 cm high, 6-20 mm in diameter. Externally milky white to pale yellowish-white. Outer scale leaves 2, plump, uniform in size or the large scale leaf embracing the smaller one, apex slightly flat or dented, frequently slightly split, the scale leaf in the centre smaller, its bottom flat. Texture hard and fragile, fracture starchy. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

The upper epidermis of scale leaves consists of 4-8 layers of cells, the lower one of 2-7 layers, their cell wall slightly thickened, some with sinuate protuberance. Crystals of calcium oxalate are visible in epidermal cells. Vascular bundles small, vessels scattered in the parenchyma tissue. Parenchyma cells filled with starch granules (Fig. 2).

Powder

Colour whitish. Starch granules simple, ovoid to triangular-ovoid or short-claviform, 5-52 μm in diameter, hilum cleft-like, pointed or V-shaped at the narrow end, striations dense and distinct. Compound and simple starch granules with 2-4 hila are rare. Stomata depressed-rounded, 40-50 μm in diameter, with 4-6 subsidiary cells. Epidermal cells subrectangular, sub-square or strip-shaped in surface view, 80-195 μm long and 21-47 μm wide, anticlinal wall slightly crooked. Crystals of calcium oxalate minute, polychromatic under the polarized microscope (Fig. 3).

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

Standard solutions

Peimisine standard solution

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

Pingbeimine A standard solution

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

Developing solvent system

Prepare a mixture of ammonium hydroxide (25%, v/v), methanol and ethyl acetate (0.5:1:6, v/v).

Spray reagent

Weigh 0.2 g of vanillin and dissolve in 10 mL of sulphuric acid.

Test solution

Weigh 10.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ammonium hydroxide (25%, v/v) and 30 mL of a mixture of dichloromethane and methanol (4:1, v/v). Sonicate (200 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

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 peimisine standard solution (2 μL), pingbeimine A 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 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 105°C for about 1 min. Examine the plate under UV light (366 nm). 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 peimisine and pingbeimine A.

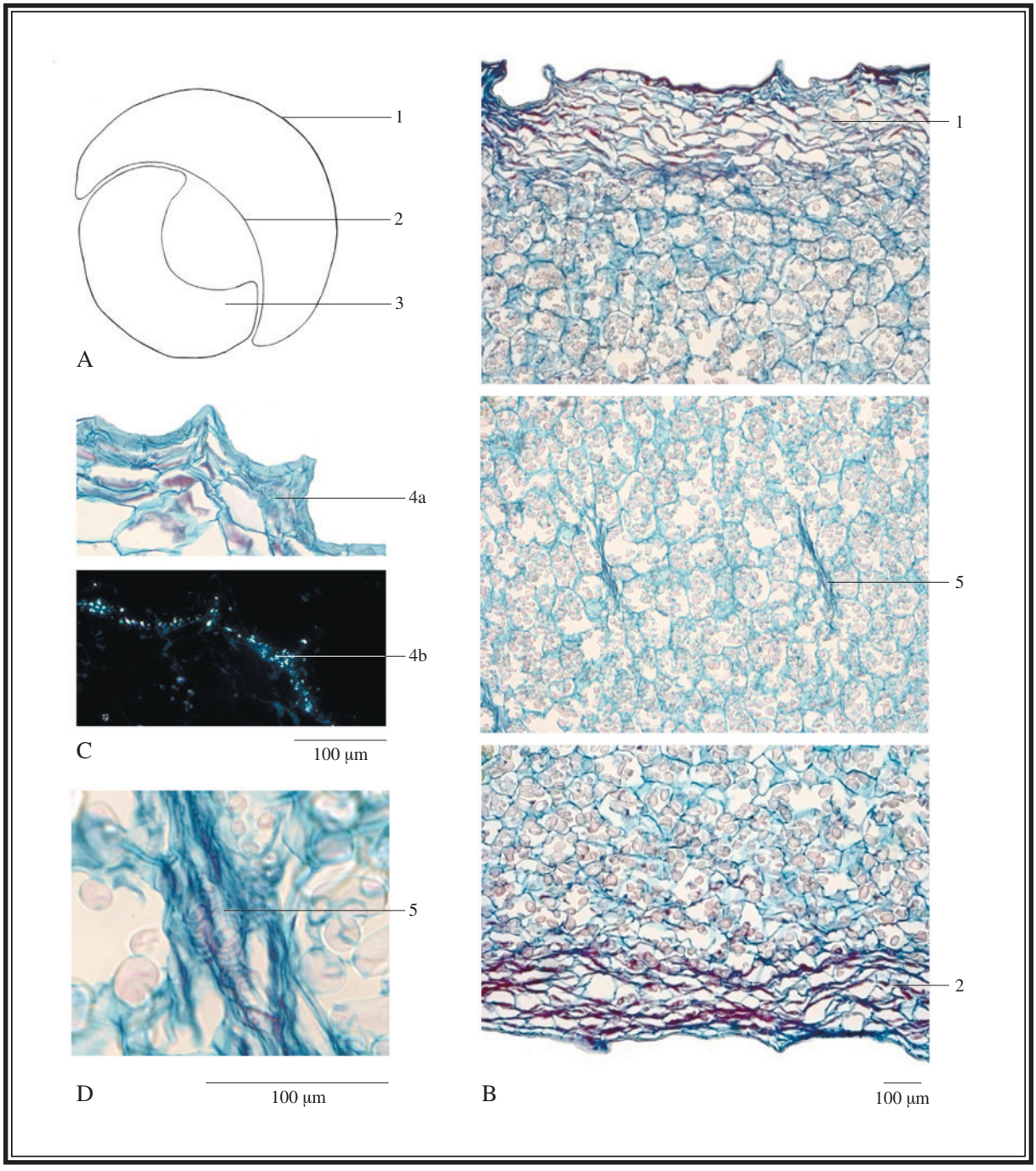


Figure 2 Microscopic features of transverse section of Bulbus Fritillariae Ussuriensis

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

1. Upper epidermis 2. Lower epidermis 3. Scale leaf 4. Crystals of calcium oxalate
5. Vessels

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

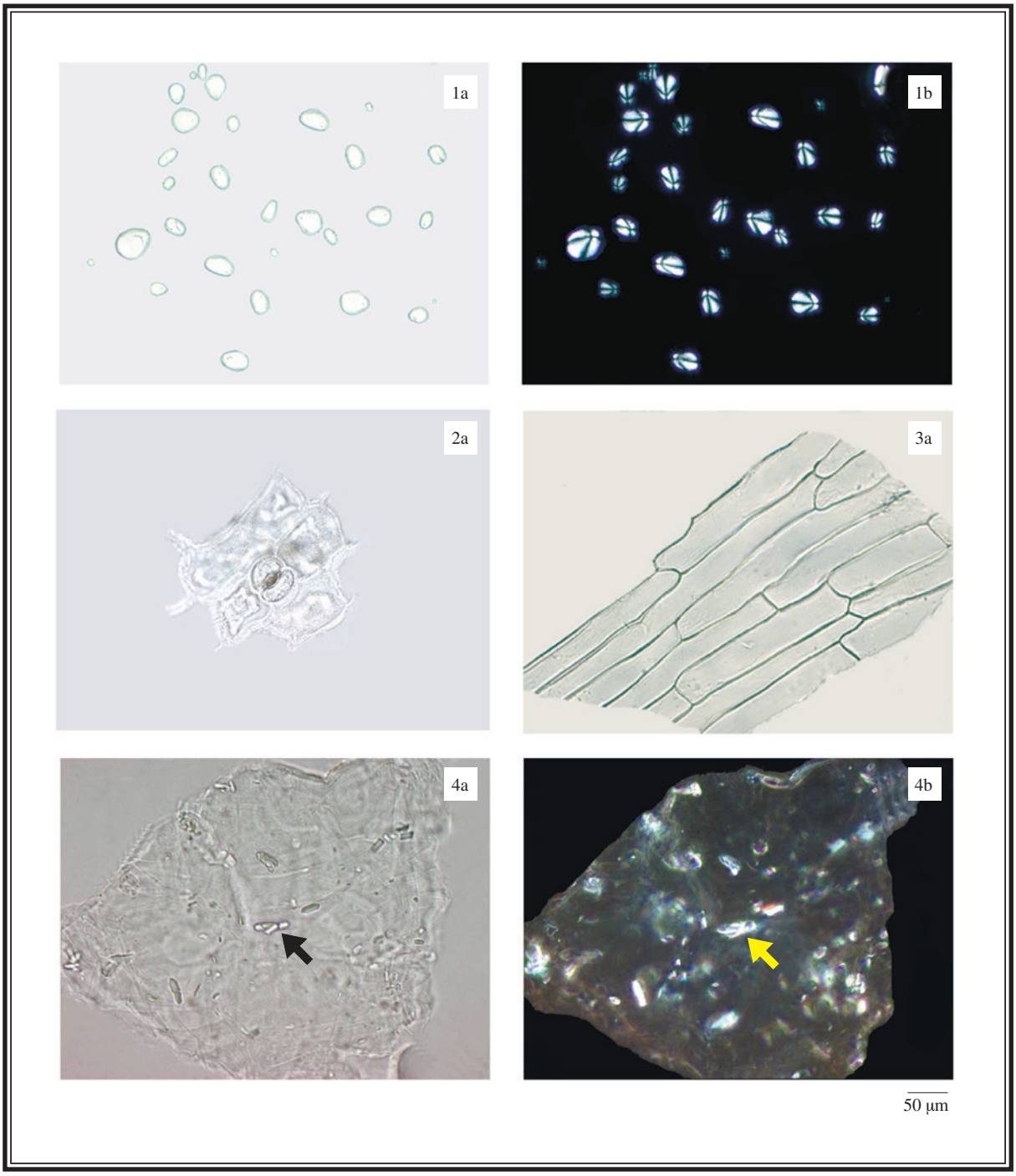
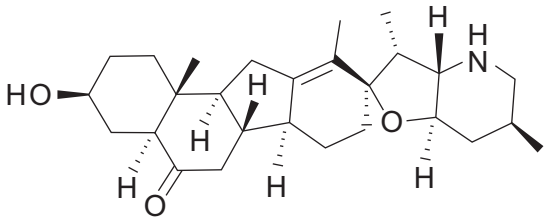


Figure 3 Microscopic features of powder of Bulbus Fritillariae Ussuriensis

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

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

(i)



(ii)

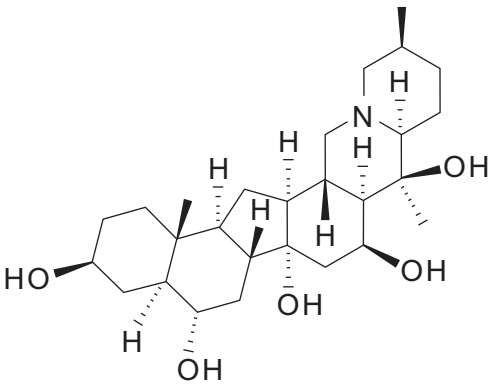


Figure 4 Chemical structures of (i) peimisine (ii) pingbeimine A

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

Standard solution

Peimisine standard solution for fingerprinting, Std-FP (200 mg/L)

Weigh 1.0 mg of peimisine CRS and dissolve in 5 mL of methanol.

Test solution

Weigh 5.0 g of the powdered sample and place it in a 200-mL round-bottomed flask, then add 10 mL of ammonium hydroxide (25%, v/v). Allow to stand for 1 h. Add 100 mL of dichloromethane. Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 200-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-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 an ELSD [drift tube temperature:105˚C; nebulizer gas (N_2) flow: 2.8 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)	0.1% Triethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	70→65	30→35	linear gradient
10–32	65→56	35→44	linear gradient
32–60	56→45	44→55	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of peimisine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of peimisine should not be more than 5.0%; the RSD of the retention time of peimisine peak should not be more than 2.0%; the column efficiency determined from peimisine peak should not be less than 20000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject peimisine Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of peimisine peak in the chromatogram of peimisine Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify peimisine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of peimisine Std-FP. The retention times of peimisine 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 six characteristic peaks of Bulbus Fritillariae Ussuriensis extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Bulbus Fritillariae Ussuriensis extract

Peak No.	RRT	Acceptable Range
1	0.68 (vs peak 4)	±0.04
2	0.76 (vs peak 4)	±0.03
3	0.90 (vs peak 4)	±0.05
4 (marker, peimisine)	1.00	-
5	1.22 (vs peak 4)	±0.08
6	1.29 (vs peak 5)	±0.04

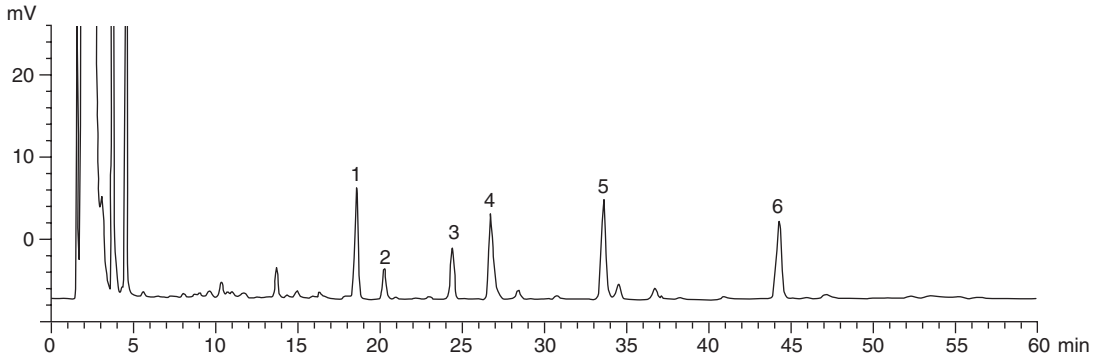


Figure 5 A reference fingerprint chromatogram of Bulbus Fritillariae Ussuriensis extract

For positive identification, the sample must give the above six 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 17.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.

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

7. ASSAY

Carry out the method as directed in Appendix XIII.

Reagents

Sodium phosphate buffer solution

Weigh 6.9 g of monobasic sodium phosphate and dissolve in 500 mL of water. Adjust the pH to 7.6 with 1 M sodium hydroxide.

Bromothymol blue solution

Weigh accurately 23.3 mg of bromothymol blue and dissolve in 100 mL of sodium phosphate buffer solution.

Standard solution

Peimisine standard stock solution, Std-Stock (500 mg/L)

Weigh accurately 5.0 mg of peimisine CRS and dissolve in 10 mL of dichloromethane.

Peimisine standard solution for assay, Std-AS

Measure accurately the volume of the peimisine Std-Stock. Transfer the solution to a 100-mL round-bottomed flask and evaporate to dryness at reduced pressure in a rotary evaporator. Pipette 6 mL of dichloromethane to dissolve the residue and produce a series of solutions of 6, 10, 14, 18, 20 mg/L for peimisine.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 1 mL of ammonium hydroxide (25%, v/v). Allow to stand for 30 min. Add 20 mL of a mixture of dichloromethane and methanol (4:1, v/v). Reflux the mixture for 2 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Pipette 6 mL of dichloromethane to dissolve the residue.

Ultraviolet / Visible spectrophotometeric system

The spectrophotometer is set at 410 nm.

Colourimetric method

Pipette 6 mL of bromothymol blue solution to the 100-mL round-bottomed flask containing the standard solution or test solution. Shake vigorously for 2 min. Transfer the mixture to a 100-mL separating funnel. Allow to stand for 2 h. Collect the dichloromethane layer in a 25-mL volumetric flask. Repeat the extraction for two more times. Combine the dichloromethane extracts. Make up to the mark with dichloromethane. Allow to stand for 25 min. Using the corresponding bromothymol blue solution as the blank. Proceed to UV/Visible analysis at 410 nm.

System suitability requirements

Perform at least five replicates of absorbance measurement, each using 0.5 mL of peimisine Std-AS (14 mg/L) by colourimetric method. The requirement of the system suitability parameter is as follows: the RSD of the absorbance of peimisine should not be more than 5.0%.

Calibration curve

Determine a series of peimisine Std-AS (0.5 mL each) in the ultraviolet / visible spectrophotometeric system and record the absorbance by colourimetric method. Plot the absorbances of peimisine against the corresponding concentrations of peimisine Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Measure the absorbance and calculate the concentration (in milligram per litre) of peimisine in the test solution, and calculate the percentage content of peimisine in the sample by using the equations indicated in Appendix XIII.

Limits

The sample contains not less than 0.15% of total alkaloids [calculated as peimisine ($C_{21}H_{41}NO_3$)], calculated with reference to the dried substance.