Lycoridis Radiatae Bulbus

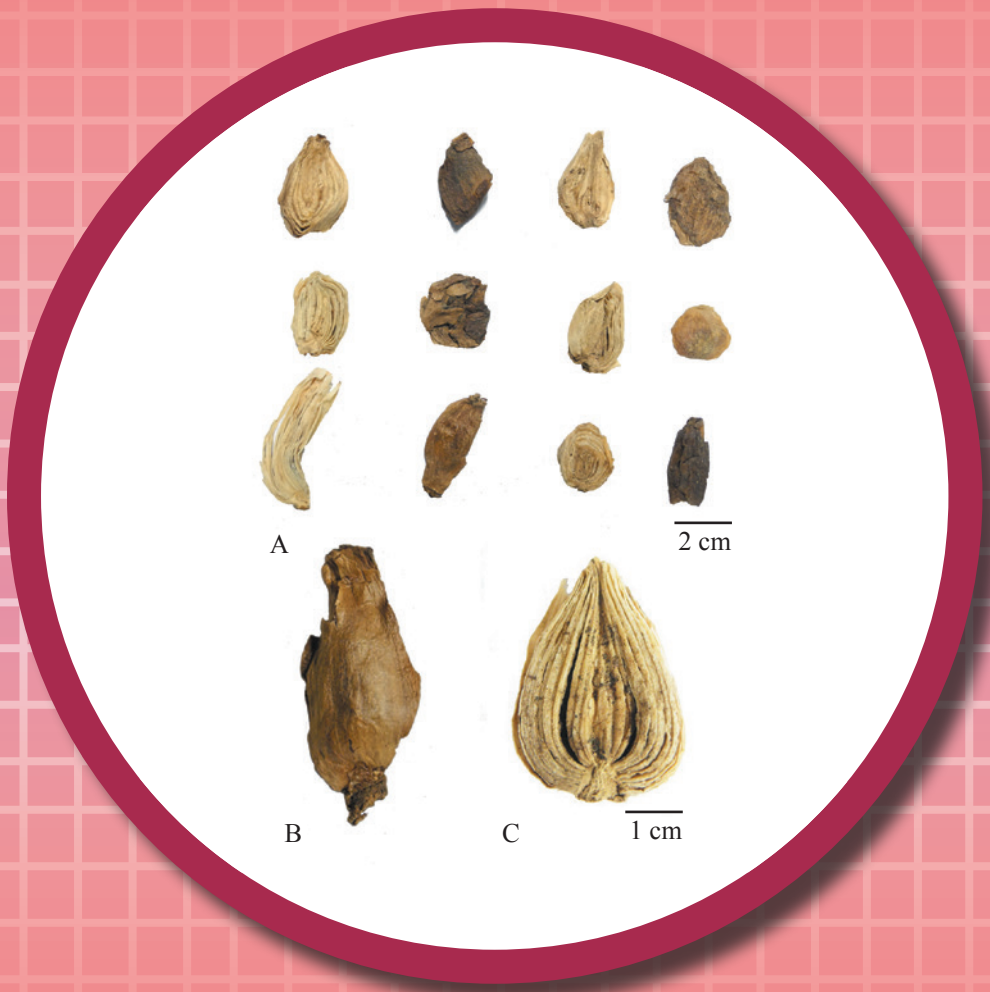

Figure 1 A photograph of Lycoridis Radiatae Bulbus

A. Lycoridis Radiatae Bulbus B. Magnified image of bulb
C. Magnified lateral view of bulb

1. NAMES

Official Name: Lycoridis Radiatae Bulbus

Chinese Name: 石蒜

Chinese Phonetic Name: Shisuan

2. SOURCE

Lycoridis Radiatae Bulbus is the dried bulb of *Lycoris radiata* (L'Hérit.) Herb. (Amaryllidaceae). The bulb is collected in autumn, washed clean, then dried under the sun to obtain Lycoridis Radiatae Bulbus.

3. DESCRIPTION

Ovoid to spherical, 3.4-5 cm long, 25-40 mm in diameter. Externally covered with 2-3 layers of dark brown dry membranous scales, easily fallen off. 10-20 layers of fleshy scales inside growing on the shortened stem plate, with yellowish-white bud in the centre. Odour characteristic, slightly irritating; taste very bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section of scale leaf

Epidermis consists of 1 layer of small parenchymatous cells. Mesophyll consists of parenchymatous cells, filled with starch granules. Mucilage cells contain raphides of calcium oxalate. Vascular bundle small, collateral, scattered in the mesophyll (Fig. 2).

Powder

Colour pale brown. Starch granules rounded or polygonal, 20-40 μm in diameter, hilum slit-shaped or stellate; black and cruciate-shaped under the polarized microscope. Mucilage cells contain raphides of calcium oxalate, crystals 100-150 μm long; polychromatic under the polarized microscope. Vessels spiral and reticulate, 12-50 μm in diameter. Fibres in bundle; yellowish-brown under the polarized microscope (Fig. 3).

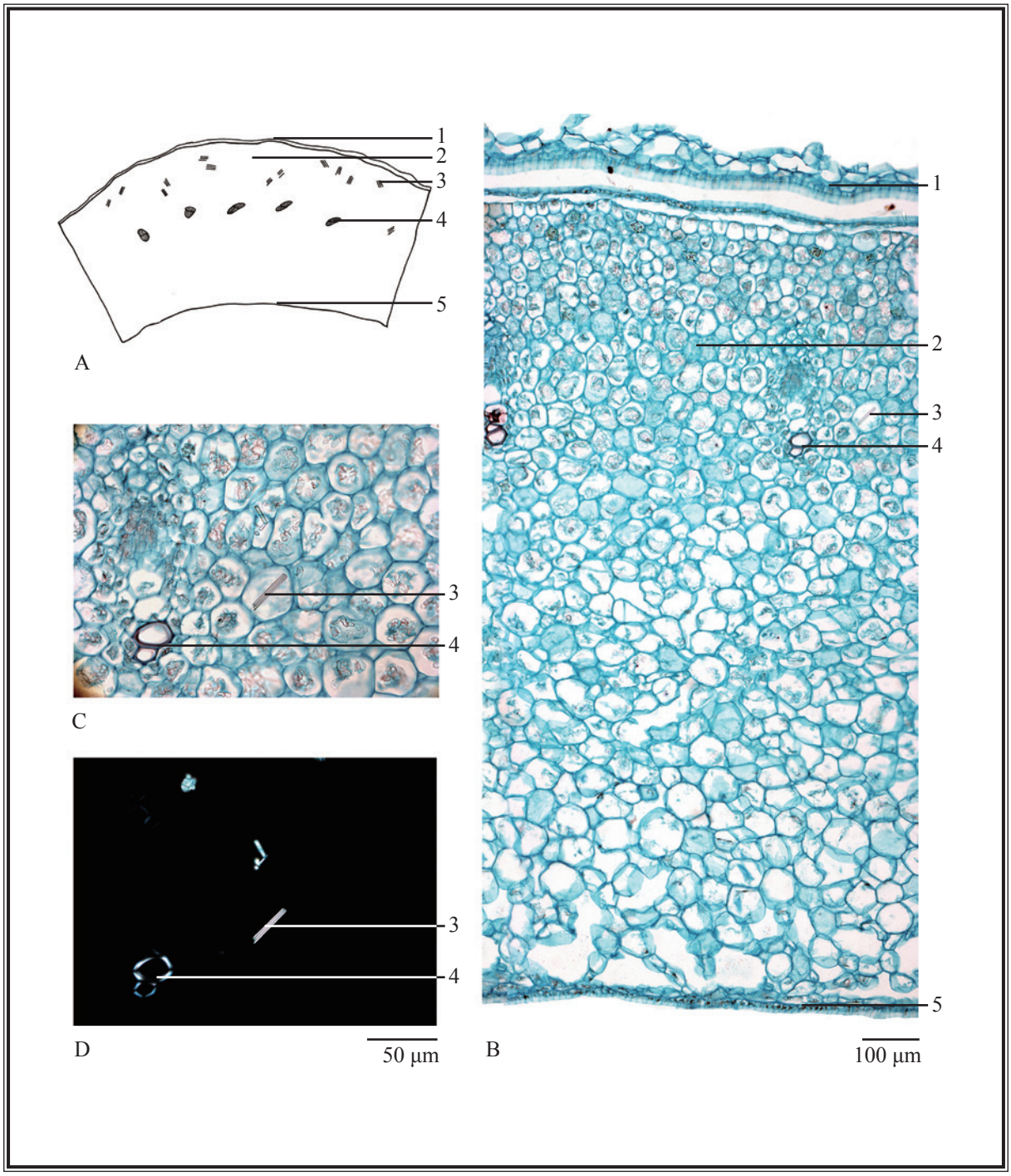

Figure 2 Microscopic features of transverse section of scale leaf of Lycoridis Radiatae Bulbus

A. Sketch B. Section illustration

C. Raphides of calcium oxalate (under the light microscope)

D. Prisms of calcium oxalate (under the polarized microscope)

1. Outer epidermis 2. Mesophyll 3. Raphides of calcium oxalate

4. Vascular bundle 5. Inner epidermis

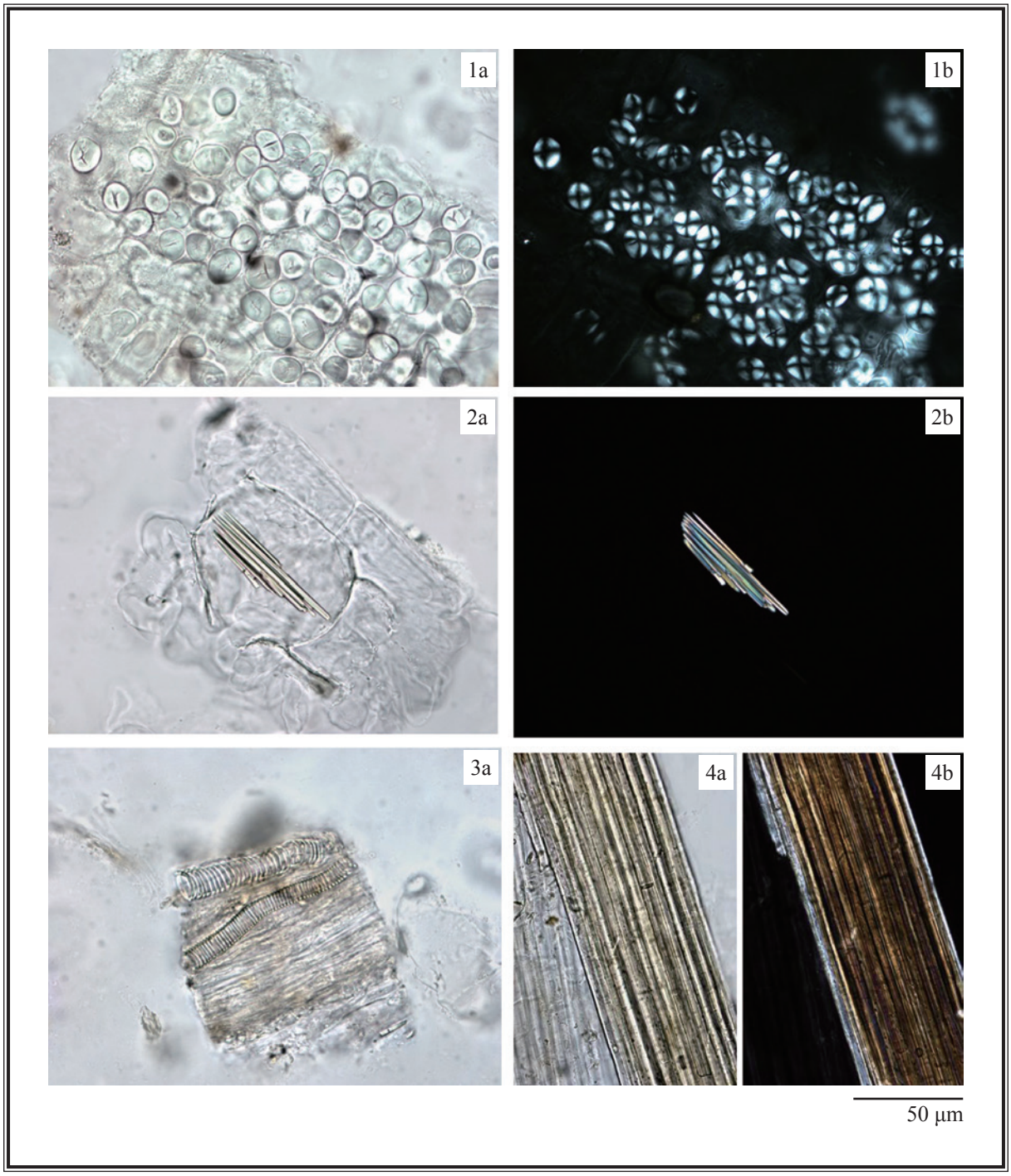

Figure 3 Microscopic features of powder of Lycoridis Radiatae Bulbus

1. Starch granules 2. Raphides of calcium oxalate in mucilage cell 3. Vessels 4. Fibres

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

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

Standard solutions

Galantamine standard solution

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

Lycorine hydrochloride standard solution

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

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), methanol, *n*-hexane and dichloromethane (0.2:1:2:7, v/v). Use the lower layer.

Staining reagent

Iodine.

Test solution

Weigh 4.0 g of the powdered sample and place it in a 50-mL conical flask, then add 6 mL of ammonium hydroxide solution (25%, v/v) and 30 mL of dichloromethane. Sonicate (100 W) the mixture for 1 h. 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 methanol. 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 galantamine standard solution (2 μL), lycorine hydrochloride standard solution (2 μL) and the test solution (7 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 7.5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Fumigate the plate with iodine vapour chamber for about 20 min until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

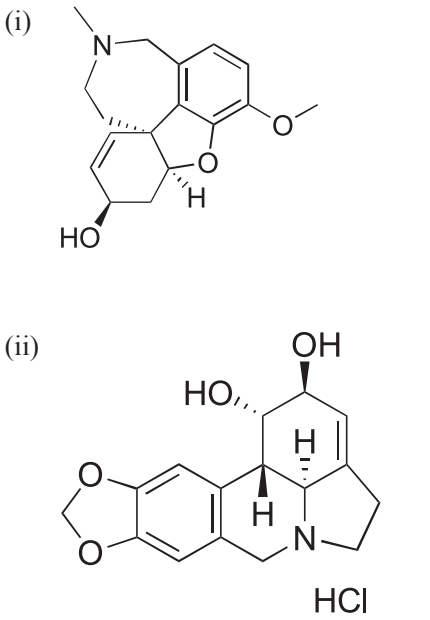

Figure 4 Chemical structures of (i) galantamine and (ii) lycorine hydrochloride

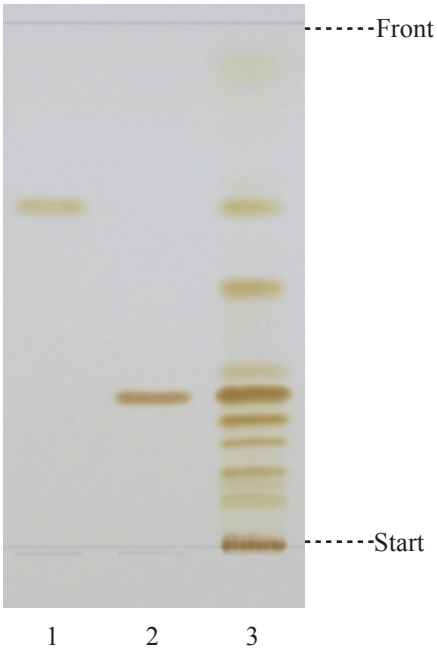

Figure 5 A reference HPTLC chromatogram of Lycoridis Radiatae Bulbus extract observed under visible light after staining

1. Galantamine standard solution 2. Lycorine hydrochloride standard solution
3. Test solution

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 galantamine and lycorine (Fig. 5).

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

Reagent

0.1 M hydrochloric acid solution

Pipette 8.33 mL of hydrochloric acid to a 1000-mL volumetric flask and make up to the mark with water.

Standard solutions

Galantamine standard solution for fingerprinting, Std-FP (500 mg/L)

Weigh 1.0 mg of galantamine CRS and dissolve in 2 mL of methanol.

Lycorine hydrochloride standard solution for fingerprinting, Std-FP (600 mg/L)

Weigh 1.2 mg of lycorine hydrochloride CRS and dissolve in 2 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 3 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 40 mL of a mixture of dichloromethane and methanol (4:1, v/v). Sonicate (100 W) the mixture for 1.5 h. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Wash the residue with a mixture of dichloromethane and methanol (4:1, v/v). Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 6 mL of formic acid (2%, v/v). Pre-condition a Mixed-Mode cation-exchange (MCX) solid-phase extraction column (3 mL, 60 mg) with 3 mL of methanol, allow to stand for 10 min, then followed by 3 mL of water. Load the sample solution to the SPE column. Add 2 mL of 0.1 M hydrochloric acid solution followed by 2 mL of methanol to the pre-conditioned column and discard the eluant. Add 3 mL of a mixture of ammonium hydroxide solution (25%, v/v) and methanol (1:4, v/v) to the column. Collect the eluant and evaporate the solvent to dryness under nitrogen. Dissolve the residue in methanol. Transfer the solution to a 1-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 (289 nm) 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)	0.05% Diethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	92	8	isocratic
10 – 12	92 → 87	8 → 13	linear gradient
12 – 30	87	13	isocratic
30 – 50	87 → 80	13 → 20	linear gradient
50 – 60	80	20	isocratic
60 – 70	80 → 68	20 → 32	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of galantamine Std-FP and lycorine hydrochloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of galantamine and lycorine should not be more than 5.0%; the RSD of the retention times of galantamine and lycorine peaks should not be more than 2.0%; the column efficiencies determined from galantamine and lycorine peaks should not be less than 60000 and 15000 theoretical plates respectively.

The *R* value between peak 2 and the closest peak; and 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 galantamine Std-FP, lycorine hydrochloride Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of galantamine and lycorine peaks in the chromatograms of galantamine Std-FP, lycorine hydrochloride Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify galantamine and lycorine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of galantamine Std-FP and lycorine hydrochloride Std-FP. The retention times of galantamine and lycorine peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Lycoridis Radiatae Bulbus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Lycoridis Radiatae Bulbus extract

Peak No.	RRT	Acceptable Range
1	0.60	± 0.03
2 (marker, lycorine)	1.00	-
3	1.20	± 0.03
4	1.42	± 0.05
5 (galantamine)	1.77	± 0.07

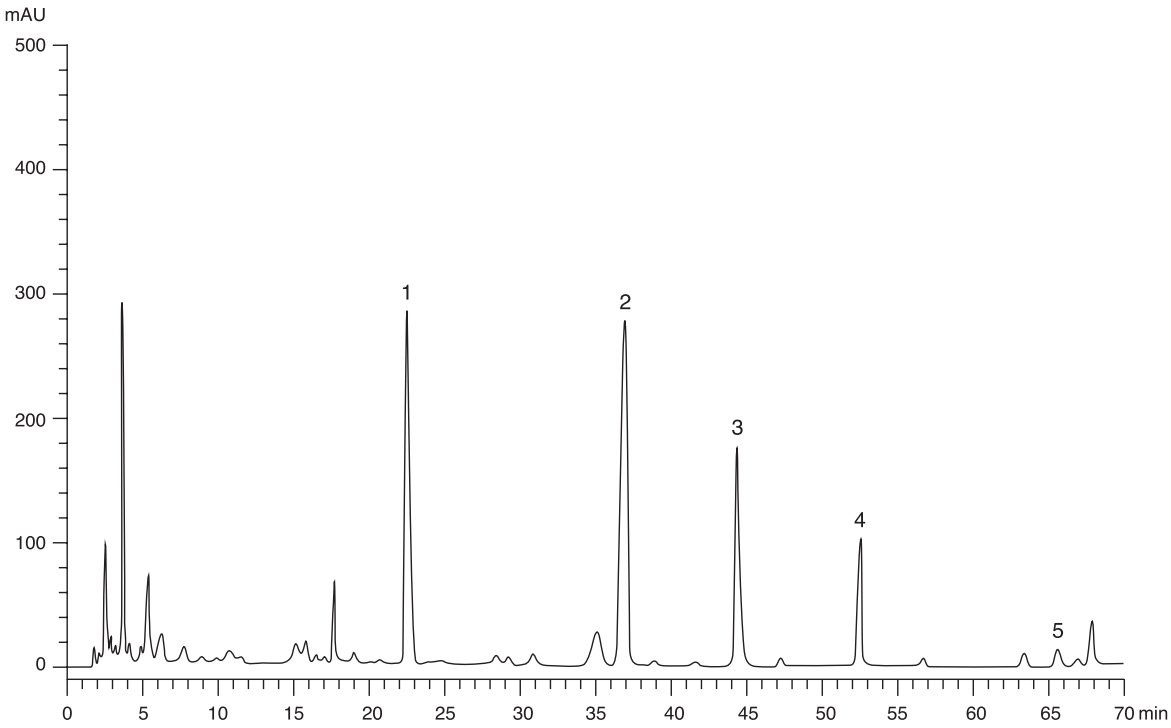

Figure 6 A reference fingerprint chromatogram of Lycoridis Radiatae Bulbus extract

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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 5.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 48.0%.

Ethanol-soluble extractives (hot extraction method): not less than 30.0%.

7. ASSAY

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

7.1 Assay of Galantamine

Standard solution

Galantamine standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 5.0 mg of galantamine CRS and dissolve in 5 mL of methanol.

Galantamine standard solution for assay, Std-AS

Measure accurately the volume of the galantamine Std-Stock, dilute with methanol to produce a series of solutions of 50, 100, 400, 600, 1000 mg/L for galantamine.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 3 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 20 mL of a mixture of dichloromethane and methanol (4:1, v/v). Sonicate (100 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction for two more times, each with 20 mL of a mixture of dichloromethane and methanol (4:1, v/v). Wash the residue with a mixture of dichloromethane and methanol (4:1, v/v). Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 1-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 (232 nm) 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.05% Diethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	90	10	isocratic
5 – 20	90 → 74	10 → 26	linear gradient
20 – 35	74	26	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of galantamine Std-AS (400 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of galantamine should not be more than 5.0%; the RSD of the retention time of galantamine peak should not be more than 2.0%; the column efficiency determined from galantamine peak should not be less than 65000 theoretical plates.

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

Limits

The sample contains not less than 0.015% of galantamine ($C_{17}H_{21}NO_3$), calculated with reference to the dried substance.

7.2 Assay of Lycorine

Standard solution

Lycorine hydrochloride standard stock solution, Std-Stock (2000 mg/L)

Weigh accurately 10.0 mg of lycorine hydrochloride CRS and dissolve in 5 mL of methanol.

Lycorine hydrochloride standard solution for assay, Std-AS

Measure accurately the volume of the lycorine hydrochloride Std-Stock, dilute with methanol to produce a series of solutions of 200, 400, 800, 1200, 2000 mg/L for lycorine hydrochloride.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 3 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 20 mL of a mixture of dichloromethane and methanol (4:1, v/v). Sonicate (100 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Repeat the extraction for two more times, each with 20 mL of a mixture of dichloromethane and methanol (4:1, v/v). Wash the residue with a mixture of dichloromethane and methanol (4:1, v/v). Combine the solutions. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 1-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 (289 nm) 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 4) –

Table 4 Chromatographic system conditions

Time (min)	0.05% Diethylamine (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	92	8	isocratic
10 – 12	92 → 87	8 → 13	linear gradient
12 – 30	87	13	isocratic
30 – 50	87 → 80	13 → 20	linear gradient
50 – 60	80	20	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of lycorine hydrochloride Std-AS (800 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of lycorine should not be more than 5.0%; the RSD of the retention time of lycorine peak should not be more than 2.0%; the column efficiency determined from lycorine peak should not be less than 28000 theoretical plates.

The *R* value between lycorine 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 lycorine hydrochloride Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of lycorine against the corresponding concentrations of lycorine hydrochloride 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 lycorine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of lycorine hydrochloride Std-AS. The retention times of lycorine 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 lycorine hydrochloride in the test solution, and calculate the percentage content of lycorine (the percentage content of lycorine hydrochloride × 0.89, where 0.89 is the molar mass ratio of lycorine and lycorine hydrochloride) in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.037% of lycorine ($C_{16}H_{17}NO_4$), calculated with reference to the dried substance.