Figure 33　A photograph of Rhizoma Alismatis

1. NAMES

Official Name: Rhizoma Alismatis

Chinese Name: 澤瀉

Chinese Phonetic Name: Zexie

2. SOURCE

Rhizoma Alismatis is the dried tuber of *Alismatis orientalis* (Sam.) Juzep. (Fam. Alismataceae). The tuber is collected in winter when the stem withers, washed clean, dried, and the fibrous roots and coarse outer tissue removed to obtain Rhizoma Alismatis.

3. DESCRIPTION

Subspherical, ellipsoid or ovoid, 2–8 cm in length and 20–70 mm in diameter. Externally yellowish-white or yellowish-brown, with irregular transverse-annular shallow furrows and numerous fibrous root scars, occasionally tuberculate bud scars attached to the base. Texture compact, fracture yellowish-white, starchy, with numerous small pores. Odour, slight; taste, slightly bitter. (Fig. 33)

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Sometimes residual cortex observed. Endodermis composed of a row of thick-walled cells, which is slightly or fully lignified. Vascular bundles mainly amphivasal. Oil-secretory cavities suborbicular, 48–214 μm in diameter. (Fig. 34)

Powder

Yellowish or yellowish-brown. Numerous starch grains present in parenchymatous cells; simple granules long-ovoid, subspherical or ellipsoid, 2–17 μm in diameter, with V-shaped, shortly slit-shaped or Y-shaped hilum; compound granules of 2–3 units; showing a black, cross-shape when

examined under a polarizing microscope. Parenchymatous cells suboribicular, some contain elliptical pits crowded into pitted areas. Anticlinal walls of endodermis cells sinuous, relatively thick, lignified, with sparse and minute pit-canals. Oil-secretory cavities mostly broken, intact ones suborbicular, 48–214 μm in diameter. Vessels mainly spiral, scalariform, and reticulate. Fibres thick-walled. (Fig. 35)

4.2 Physicochemical Identification

Procedure

Weigh 0.25 g of the powdered sample and put into a 100-mL conical flask, then add 10 mL of dichloromethane. Sonicate the mixture for 30 min. Filter and transfer 0.5 mL of the filtrate to a test tube. Cautiously add about 0.5 mL of sulphuric acid along the inner wall of the tube. Allow to stand for about 20 min. A reddish-brown or yellowish-brown layer is observed in the interface of the two solvent layers.

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

Standard solutions

Alisol B standard solution

Weigh 1.0 mg of alisol B and dissolve in 2 mL of methanol.

Alisol B monoacetate standard solution

Weigh 1.0 mg of alisol B monoacetate and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (40–60 ˚C) and ethyl acetate (8:9, v/v).

Spray reagent

Mix 1 mL of dilute sulphuric acid (50%, v/v) and 10 mL of *p*-hydroxybenzaldehyde in methanol (2%, w/v). Freshly prepare the reagent.

Test solution

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 30 mL of methanol. Sonicate the mixture for 30 min, filter and then evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol. Pass the solution through a clean-up column (20 x 200 mm) packed with 15.0 g of RP-18 silica gel (40–60 μm) pre-washed with 100 mL of methanol (60%). Wash the column with 200 mL of methanol (60%). Elute the column with 200 mL of methanol (80%). Collect the eluate and evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 0.5 mL of methanol.

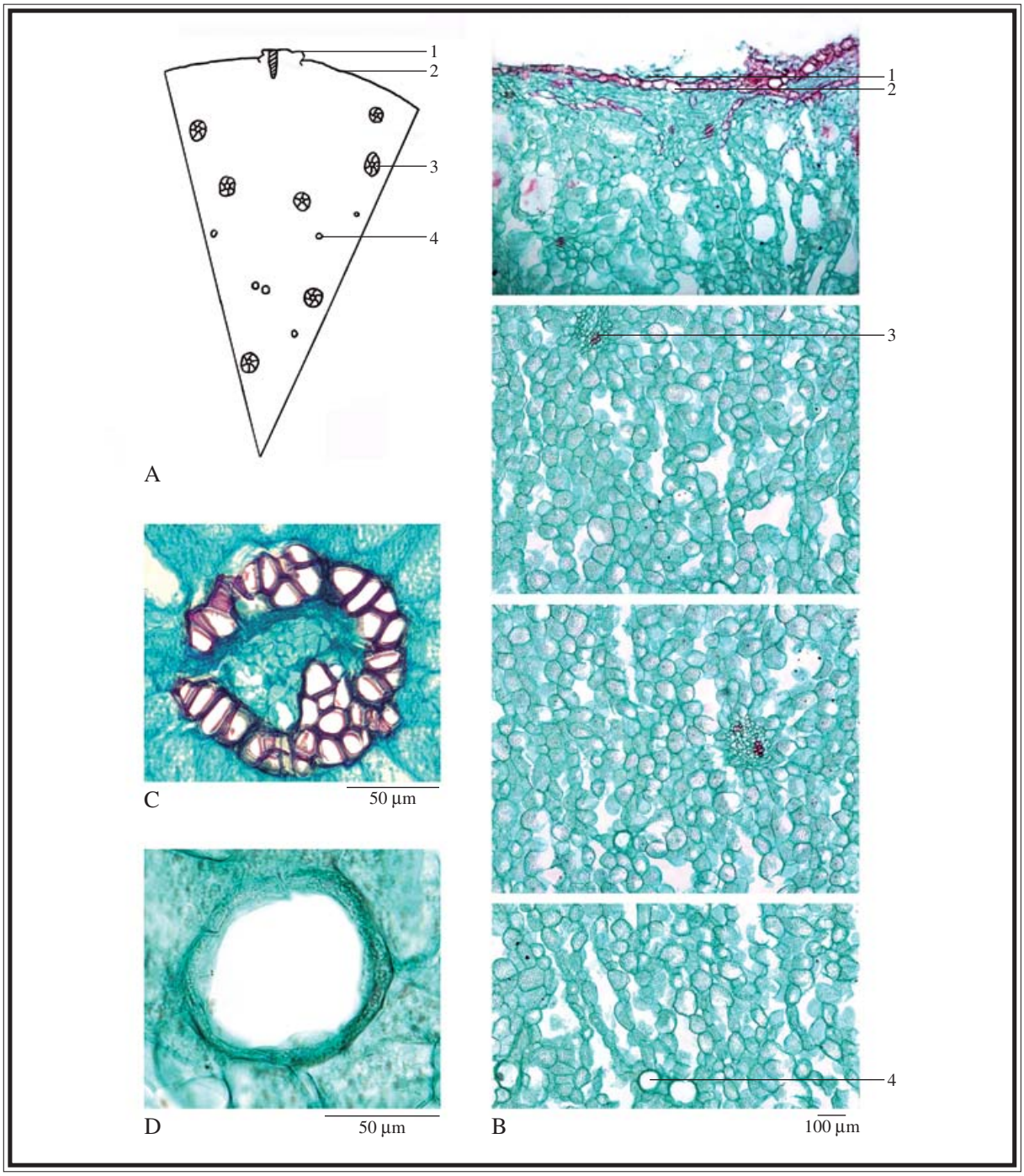

Figure 34 Microscopic features of transverse section of Rhizoma Alismatis

A. Sketch B. Section illustration C. Vascular bundle D. Oil-secretory cavity

1. Cortex 2. Endodermis 3. Vascular bundle 4. Oil-secretory cavity

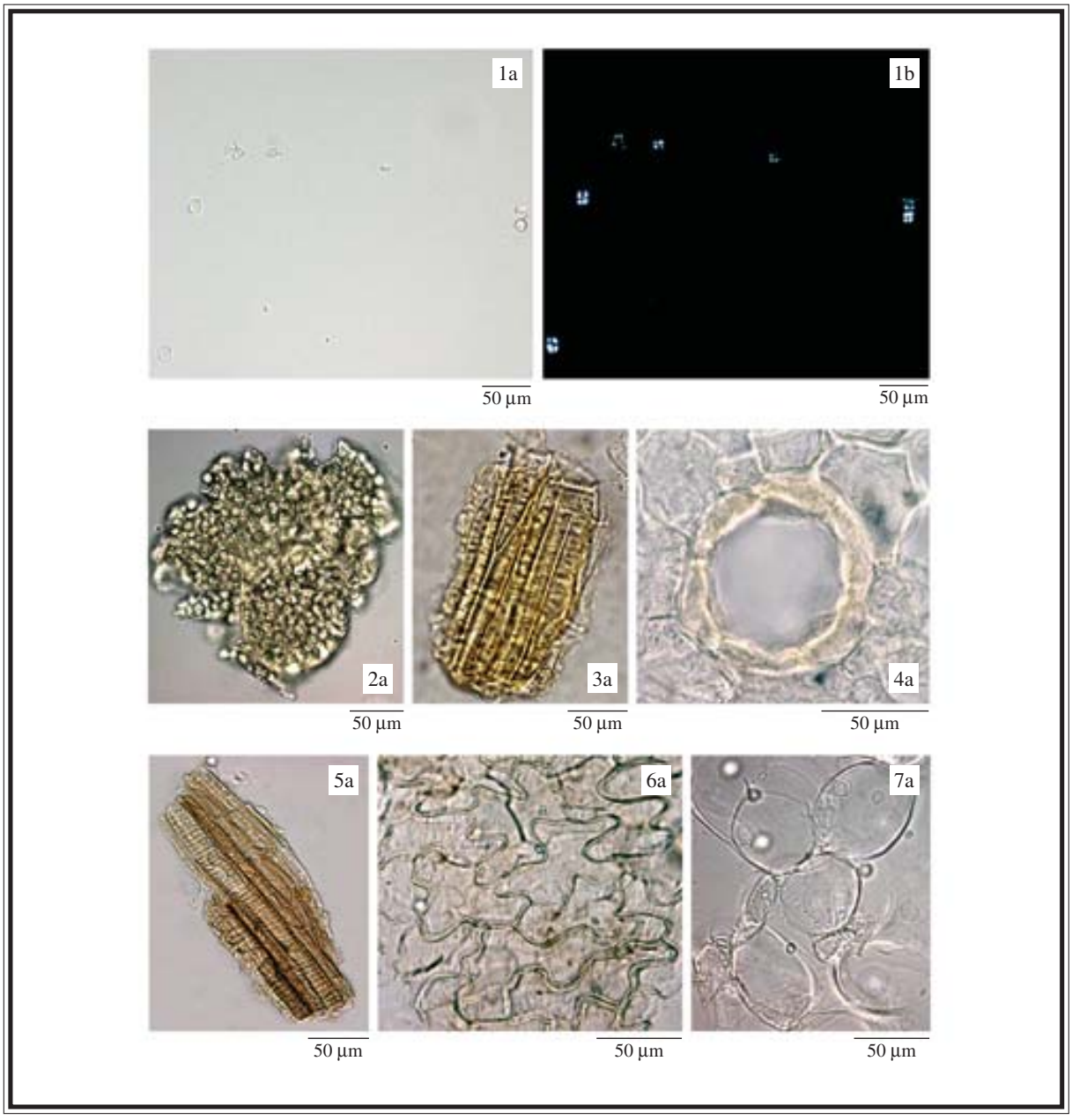

Figure 35 Microscopic features of powder of Rhizoma Alismatis

1. Starch grains 2. Starch grains in parenchymatous cells 3. Fibres 4. Oil-secretory cavity
5. Vessels 6. Endodermis cells 7. Parenchymatous cells

a. Features under a light microscope b. Features under a polarizing microscope

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Develop over a path of about 5 cm. Apply separately alisol B standard solution, alisol B monoacetate standard solution and the test solution (4 µL each) to the plate. 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 80 ˚C for about 10 min. Examine the plate in 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 alisol B and alisol B monoacetate.

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

Standard solution

Alisol B monoacetate standard stock solution, Std-Stock (1000 mg/L)

Weigh 1.0 mg of alisol B monoacetate and dissolve in 1 mL of methanol.

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

Pipette 0.5 mL of alisol B monoacetate Std-Stock into a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol and weigh. Sonicate the mixture for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x *g* for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 nm) and a column (3.9 x 300 mm) packed with ODS bonded silica gel (4 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	100	0	isocratic
10–45	100 ➔ 0	0 ➔ 100	linear gradient
45–60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 20 μL of alisol B monoacetate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of alisol B monoacetate should be not more than 3.0%; the RSD of the retention time of alisol B monoacetate peak should be not more than 2.0%; the column efficiency determined from alisol B monoacetate peak should be not less than 200,000 theoretical plates.

The *R* value between alisol B peak and alisol B monoacetate peak (Fig. 36) in the test solution should be not less than 1.5.

Procedure

Separately inject alisol B monoacetate Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of alisol B monoacetate peak in the chromatogram of alisol B monoacetate Std-FP and the retention times of the four characteristic peaks (Fig. 36) in the chromatogram of the test solution. Under the same HPLC conditions, identify alisol B monoacetate peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of alisol B monoacetate Std-FP. The retention times of alisol B monoacetate 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 Rhizoma Alismatis extract are listed in Table 11.

Table 11 The RRTs and acceptable ranges of the four characteristic peaks of Rhizoma Alismatis extract

Peak No.	RRT	Acceptable Range
1	0.63	±0.03
2	0.90	±0.03
3 (alisol B)	0.95	±0.03
4 (marker, alisol B monoacetate)	1.00	-

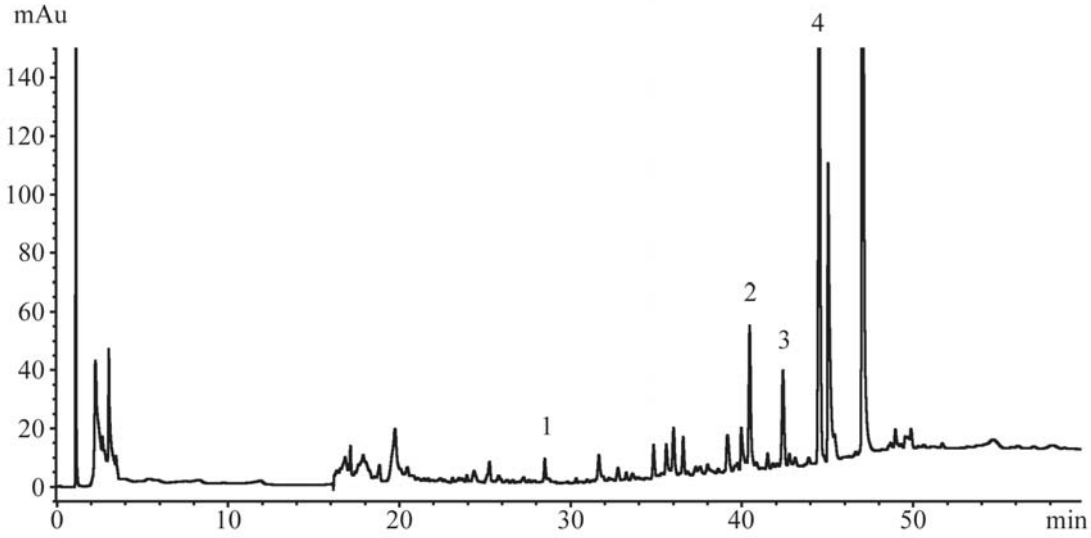

Figure 36 A reference fingerprint chromatogram of Rhizoma Alismatis extract

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. 36).

5. TESTS

5.1 **Heavy Metals** *(Appendix V)*: meet the requirements.

5.2 **Pesticide Residues** *(Appendix VI)*: meet the requirements.

5.3 **Mycotoxins – Aflatoxins** *(Appendix VII)*: meet the requirements.

5.4 **Sulphur Dioxide Residues** *(Appendix XIV)*: meet the requirements.

5.5 **Foreign Matter** *(Appendix VIII)*: not more than 1.0%.

5.6 **Ash** *(Appendix IX)*

Total ash: not more than 5.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 **Water Content** *(Appendix X)*: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 17.0%.
Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

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

Standard solution

Alisol B monoacetate standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of alisol B monoacetate and dissolve in 10 mL of methanol.

Alisol B monoacetate standard solution for assay, Std-AS

Measure accurately the volume of alisol B monoacetate Std-Stock, dilute with methanol to produce a series of solutions of 10, 50, 100, 200, 400 mg/L for alisol B monoacetate.

Test solution

Weigh accurately 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add accurately 20 mL of methanol and weigh. Sonicate for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 nm) and a column (3.9 x 300 mm) packed with ODS silica bonded gel (4 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0–10	100	0	isocratic
10–45	100 ➔ 0	0 ➔ 100	linear gradient
45–60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 5 μL of alisol B monoacetate Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of alisol B

monoacetate should be not more than 5.0%; the RSD of the retention time of alisol B monoacetate peak should be not more than 2.0%; the column efficiency determined from alisol B monoacetate peak should be not less than 200,000 theoretical plates.

The *R* value between alisol B monoacetate peak and the closest peak in the test solution should be not less than 1.5.

Calibration curve

Inject a series of alisol B monoacetate Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of alisol B monoacetate against the corresponding concentrations of alisol B monoacetate Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify alisol B monoacetate peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of alisol B monoacetate Std-AS. The retention times of alisol B monoacetate 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 alisol B monoacetate in the test solution, and calculate the percentage content of alisol B monoacetate in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.082% of alisol B monoacetate ($C_{32}H_{50}O_5$), calculated with reference to the dried substance.